IMPROVED GROWTH, YIELD, AND NUTRITIONAL QUALITY OF CULINARY HERBS AND LEAFY GREENS VIA MANIPULATION OF LIGHTING ENVIRONMENT IN INDOOR VERTICAL FARMS

A Dissertation

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ABSTRACT

There are increasing interests to produce culinary herbs and leafy greens in indoor vertical farms (IVFs) due to increasing world population, resource competition, and unusual climate. Light is one of the most important environmental factors, which affects plant photosynthesis, morphology, yield, and secondary metabolism. Advancement of light emitting diodes technology provides researchers the opportunity to optimize lighting conditions in IVFs to improve plant productivity and quality. Therefore, the objective of the present study is to improve plant growth, yield, and nutritional quality in culinary herbs and leafy greens via manipulating the lighting environment in IVFs.

Five experiments were conducted in a growth room using green and purple/red basil (*Ocimum basilicum*) and four *Brassica* species. Results indicated that higher daily light integrals of 12.9 to 17.8 $mol \cdot m^{-2} \cdot d^{-1}$ improved plant photosynthesis, yield, and phytochemical accumulation in green basil plants. In combined red and blue (R&B) light, increases of blue light proportions increased plant photosynthesis, chlorophyll content, and phytochemical concentrations in basil and *Brassica* species, while plants grown under higher red light proportions had increased stem elongation, leaf expansion, and greater plant yield. Addition of green light to R&B light decreased photosynthesis, chlorophyll content, and yield in all tested plant species. Substituting red or blue light with green light increased plant photosynthesis in the lower leaves in purple basil plants, but showed no effects in green basil plants. Phytonutrients accumulation in green basil plants decreased by substituting blue or R&B light with green light, while decreased in purple basil plants

by substituting red or R&B light with green light. Substituting photosynthetically active radiation light with far-red light increased plant stem and petiole elongation and shoot FW by 6%-23% in green basil plants, which also resulted in increased phytochemical concentrations and antioxidant capacity. Supplemental ultraviolet-B (UV-B) radiation increased phytochemical concentrations up to 169% in green basil leaves but decreased plant yield, while lower UV-B radiation doses increased antioxidant capacity in *Brassica* species without yield reduction. In conclusion, this study unveils how plants respond to changes of light intensity, quality, and supplemental UV-B radiation, providing useful information for light source selection in IVFs.

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CHAPTER I

INTRODUCTION

1.1 Introduction

The demand for more resources (e.g. land, water, and energy) to produce more food is ever increasing with the population growth worldwide, which is expected to reach 9.3 billion by 2050. However, our food production capacity is increasingly threatened by global climate change and competition of resources such as arable land, clean water, and fuel energy (Dunwoody, 2014; Liaros et al., 2016). Meanwhile, with the development of urbanization, the food demand is mainly in the urban area with 68% of the population living in an urban environment, which increases costs of long-distance transportation and decreases the quality of food products (Kozai et al., 2015). The negative impacts of the conventional food production systems are exacerbating, including groundwater contamination from pesticide and synthetic nitrogen use, soil erosion and degradation, a large volume of greenhouse gas emissions and persistently high levels of food insecurity and disease (Cleveland et al., 2015). As a consequence, the challenge facing agriculture in the upcoming 50 years will be an increasing demand for food to feed ever larger cities with ever fewer resources. In this scenario, an increasing interest has been placed on controlled environment agriculture (CEA), especially indoor vertical farms (IVFs), also called plant factory with artificial lighting, used as an alternative production system to conventional open field production (Castilla and Hernandez, 2006; Despommier, 2013; Kozai, 2013).

Indoor vertical farm refers to a plant production facility with a thermally insulated and nearly airtight warehouse-like structure, using multi-layer cultivation shelves installed with artificial lighting (Kozai, 2013). Compared to open field production, IVFs exhibit social, economic, and environmental sustainability with many advantages. (1) IVFs can achieve year-round production with complete environment control over light, temperature, relative humidity, CO_2 concentration, and nutrients regardless of local weather conditions (Kozai, 2007; Kozai, 2013). Meanwhile, plant yield, nutritional quality, and harvesting time could be regulated depending on the marketing requirements owning to the accurate manipulation and control of the environmental conditions. (2) Resource utilization efficiencies of water, land, and fertilizer in IVFs are improved significantly compared to open field production (Kozai, 2007; Ohyama et al., 2003; Yokoi et al., 2005). For example, IVFs use less than 5% water compared to open field production (Ohyama et al., 2003). (3) Sustainable production is achieved in IVFs with less resource consumption (land, water, and CO_2) and less emission of environmental pollutants (pesticides and chemicals) (Kozai, 2012). (4) IVFs could be placed close to the consumer which significantly reduces the cost and time involved in food packing and transportation, as well as the loss of crop quality and quantity due to long distance transportation (Ohyama et al., 2008; Pessu et al., 2011). (5) Working environment for farmers in IVFs is more comfortable, and placement of IVFs in cities will increase job opportunity in urban areas.

With all these advantages, the crop production in IVFs is currently limited to high value crops with short production periods, such as specialty leafy vegetables, micro/baby greens,

transplants, herbs, and medicinal plants. This limitation is the initial high construction and operation costs and immature technologies. Among these crops, culinary herbs such as basil (*Ocimum basilicum*) and leafy greens such as *Brassica* vegetables are highly diverse in species and cultivars and a valuable part of human diet owing to their nutritive values. For example, basil is called the "king of herbs" or the "royal herb" and is widely used as a culinary herb and medicinal plant due to its specific aromatic flavor and relatively high content of phenolic compounds (Chiang et al., 2005; Makri and Kintzios, 2008). *Brassica* vegetables are popularly consumed owing to their good flavor, vivid colors, and abundance in bioactive compounds, including glucosinolates, ascorbic acid, and phenolic compounds (Kopsell et al., 2003; Qian et al., 2016). With increasing research efforts to identify their health-promoting properties and potential applications, the interest in these crops continues to grow and consumer demand keeps increasing (Keservani et al., 2010; Mills and Jones Jr, 1996). The increasing demand is accompanied by issues of quality and consistency in open field production (Zobayed et al., 2005). To meet the market demand and ensure safety and high quality of these crops, increasing numbers of farmers and entrepreneurs are adopting to IVFs production.

Light is an indispensable energy for crop production and one of the largest energy consumption components in IVFs, which influences plant photosynthesis, photomorphogenesis, and phytochemical accumulation (Dou et al., 2017; Kang et al., 2013). In IVFs, artificial lighting system represents the only source of light and its features are fundamental for optimal plant performance and reduced production cost. However,

due to limited information, the effects of artificial lighting on plant growth and development and the optimal crop light requirements is still unclear to growers and researchers. To be at the forefront of this movement, our research project addressed how different lighting conditions affect culinary herbs' and leafy greens' production in IVFs.

1.2 Advantages of Light Emitting Diodes (LEDs)

Since lighting is one of the largest power consumption components in IVFs, selection of light sources can have a significant influence on the construction and operation costs, in addition to their effects on plant growth and development (Kozai, 2012). With the development of LED technology, it has become a widely used light source in IVFs owning to its high energy utilization efficiency and low surface temperature compared to the other lamp types (Bantis et al., 2018; Pennisi et al., 2019).

LEDs were initially adopted for lighting research for plant growth in mid 1980s and early 1990s by the University of Wisconsin Center for Space Automation and Robotics, NASA, and the Kennedy Space Center (Cocetta et al., 2017). Use of LEDs has gradually increased in horticultural production due to its advantages compared to other light types (Stutte, 2009). Firstly, energy use efficiency of LEDs is significantly improved, which provides high light intensity with low radiant heat and surface temperature, allowing LEDs to be installed close to the plant canopy or even intra-canopy. The electricity-to-light energy conversion factor of LEDs is around 60% higher compared to conventional fluorescent lamps (FLs) (Kozai et al., 2015). Secondly, spectra wavelength of LEDs could be customized allowing optimization of light spectra to the demand of each plant species

and cultivar (Stutte, 2009). Thirdly, they offer longer durability and higher operating capabilities such as short response time, small size, and light weight compared to other light types (Mitchell et al., 2015; Morrow, 2008; Stutte, 2015). Although there are still obstacles influencing the use of LEDs in horticulture (high cost and developing technology), the mass production, new techniques, and simplified manufacturability and maintenance would ensure its further cost reductions.

1.3 Photosensory Photoreceptors

Plant responses to light conditions are triggered by changes in light intensity, quality [wavelength distribution from ultraviolet (UV, 280-399 nm) to far-red (700-780 nm) light], direction, and duration, to modulate plant growth and development. Plants possess two types of photoreceptors, photosynthetic pigments that harvest light energy for photosynthesis, and photosensory receptors that mediate non-photosynthetic light responses. Signals from the photoreceptors can regulate the expression of genes involved in cell division and enlargement, which form various tissues such as floral buds and leaf primordia (Anpo et al., 2018). Five photosensory systems have been identified to date, including phytochromes, cryptochromes, phototropins, members of the Zeitlupe family, and UV Resistance locus 8 (Bantis et al., 2018). Understanding properties of photoreceptors and their involvements in plant responses would provide useful information during the selection of light sources to improve plant productivity and quality in IVFs.

1.3.1 Phytochromes

Phytochromes are primarily red (600-699 nm) and far-red light sensing photoreceptors, which is the first plant photoreceptor identified at the molecular level. There are two reversible forms of phytochromes, the biologically inactive P_r (for red light absorbing, peaks at 660 nm) form and active P_{fr} (for far-red light absorbing, peaks at 730 nm) form (Quail, 2002). In general, red light activation of phytochromes may be reversed by far-red light. The phytochrome photoequilibrium (PPE), which estimates the proportion of P_{fr} in total phytochromes, depends on the spectral distribution of light sources and phytochrome absorption (Sager et al., 1988).

Phytochromes family consists of five members, designated phyA to phyE, and individual members of the family have differential, albeit frequently overlapping, photosensory and/or physiological functions in controlling plant responses from seed germination to flowering initiation, which is stated in Table 1 (Li et al., 2011). For example, at least three phytochromes (phyA, phyB and phyE) are involved in the control of seed germination in arabidopsis (*Arabidopsis thaliana*). PhyA is responsible for the irreversible very low fluence responses (VLFR) triggered by a wide variety of radiations (UV, visible, and far-red light), while phyB controls the red/far-red photo-reversible low fluence responses (LFRs). However, phyE was also found to play a role in controlling seed germination in continuous far-red light. This could be either because phyE is directly involved in the photoreception of far-red light for this response, or because phyA requires phyE to mediate seed germination.

Seedling de-etiolation is initiated when seedlings emerge from the soil and perceive light radiation, which is characterized by several morphological changes, including hypocotyl growth inhibition, cotyledon expansion, and chloroplast development (Chen and Chory, 2011). PhyA is the primary photoreceptor responsible for perceiving and mediating various responses to far-red light, while phyB and phyC responds to red light, and phyB is the predominant phytochrome regulating de-etiolation in response to white and red light (Li et al., 2011; Quail, 2002).

Shade avoidance responses include elongation of stems and petioles, accelerated flowering time, and increased apical dominance, which elevate leaves toward light (Li et al., 2011). PhyB is the predominant suppressor of shade avoidance responses in high red: far-red (R:FR) ratio, as *phyB* mutants display a constitutive shade avoidance phenotype, such as elongated petiole and early flowering. Shade avoidance responses enabled by low R:FR ratios can be effectively phenocopied by end of day far-red (EOD-FR) treatment, which is regulated by phyB, phyD, and phyE.

Table 1. Different roles of phytochrome family members in seedling and early vegetative development (Li et al., 2011).

Phytochrome Members	Primary Photosensory Activities	Primary Physiological Roles
phyA	VLFRs	Seed germination under a broad spectrum of light conditions (UV, visible, FR).
	FR-HIRs	Seedling de-etiolation under FR_c; promoting flowering under LD.
phyB	LFRs	Seed germination under R_c
	R-HIRs	Seedling de-etiolation under R_c
	EOD-FR (R:FR ratio)	Shade avoidance response (petiole and internode elongation, flowering).
phyC	R-HIRs	Seedling de-etiolation under R_c
phyD	EOD-FR (R:FR ratio)	Shade avoidance response (petiole and internode elongation, flowering).
phyE	LFRs	Seed germination
	EOD-FR (R:FR ratio)	Shade avoidance response (petiole and internode elongation, flowering).

VLFRs: very-low-fluence responses; R-LFRs: red low-fluence responses; R-HIRs: red light high-irradiance responses; FR-HIRs: far-red light high-irradiance responses; FR_c: continuous far-red light; R_c: continuous red light; LD: long day light condition; EOD-FR: end-of-day far-red light; R:FR ratio: red: far-red ratio.

1.3.2 Cryptochromes

Cryptochromes are primarily blue (400-499 nm)/UV-A light photoreceptors, which work together with phytochromes to regulate various light responses, including regulation of cell elongation and photoperiodic flowering, and act together with phototropins to mediate blue light regulation of stomatal opening (Chory, 2010; Li and Yang, 2007). There are two members of cryptochromes, CRY1 and CRY2, with overlapping functions and primarily mediates blue light inhibition of hypocotyl elongation and photoperiodic control

of floral initiation, respectively. CRY1 plays a major role in blue light inhibition of hypocotyl elongation, whereas CRY2 plays a relatively minor one compared to CRY1 (Yu et al., 2010). Although phototropins are the major photoreceptor regulating stomata opening, it was found that in response to blue light, the *cry1 cry2* mutant and CRY1 overexpressing plants exhibit reduced and increased stomata opening, respectively, which indicated stimulation of stomata opening by cryptochromes (Mao et al., 2005). In addition to the light responses discussed above, cryptochromes are also found to regulate chloroplast development and stimulate anthocyanin accumulation in plants (Li and Yang, 2007; Yu et al., 2010).

1.3.3 Phototropins

Phototropins are blue/UV-A light photoreceptors controlling a range of plant responses including phototropism, light-induced stomatal opening, and chloroplast movements in response to light intensity (Christie, 2007; Zhang and Folta, 2012). Two members of phototropins, phot1 and phot2, exhibit partially overlapping roles in these regulations. For instance, both phot1 and phot2 act to regulate hypocotyl phototropism in arabidopsis plants in response to high intensities of unilateral blue light, while hypocotyl phototropism is solely mediated by phot1 under low light intensities (Pedmale et al., 2010). Phot1 and phot2 also redundantly regulate stomatal opening by mediating blue light dependent hyperpolarization of membrane potential of guard cells, allowing plants to regulate CO_2 uptake for photosynthesis and water loss through transpiration (Briggs and Christie, 2002). Meanwhile, phototropins regulate chloroplast relocation responding to different light

intensities. Under low light intensities, phot1 and phot2 induce chloroplast movement and accumulation to the upper cell surface to promote light capture for photosynthesis, while under high light intensities, chloroplasts move away from the site of radiation to prevent photodamage of the photosynthetic apparatus, which is mediated only by phot2 (Christie, 2007).

1.3.4 Members of the Zeitlupe family

Members of Zeitlupe family, including ZEITLUPE (ZTL), FLAVIN-BINDING KELCH REPEAT F-BOX 1 (FKF1), and LOV KELCH PROTEIN 2 (LKP2), is a group of blue light photoreceptors (Kevei et al., 2006; Somers et al., 2004). Zeitlupe family participates in regulating the period of circadian oscillation, photoperiodic flowering, and hypocotyl elongation (Miyazaki et al., 2015). Somers et al. (2004) reported that *fkf1* mutants have short hypocotyls under continuous blue or red light, while LKP2-overproducing plants have elongated hypocotyls under continuous blue, red, or white light. This indicated that even though Zeitlupe family consists of blue light photoreceptors, they could promote hypocotyl growth under red or white light by inhibiting the phyB mediated signal transduction pathway, as phyB is the main receptor mediating red light induced inhibition of hypocotyl elongation (Chory, 2010).

1.3.5 UV Resistance locus 8

The UV-B specific photoreceptor, UV Resistance Locus 8 (UVR8), initiates UV-B mediated signaling pathways in response to low levels of UV-B radiation. Under UV-B

radiation, UVR8 is translocated from cytosol to nucleus and interacts with COP1 (CONSTITUTIVELY PHOTOMORPHOGENIC 1), promoting the expression of HY5 (ENLONGATED HYPOCOTYL 5) and HYH (HY5 HOMOLOG) (Kaiserli, 2018). In turn, the expression of HY5 and HYH increases the expression of key elements for UV-B acclimation, including genes encoding enzymes of the phenylpropanoid pathway (Schreiner et al., 2012). Meanwhile, perception of low UV-B radiation by UVR8 also affects plant morphology, causing growth retardation such as the inhibition of hypocotyl elongation (Jansen and Bornman, 2012). Recently, UVR8 was also shown to be involved in regulating thermomorphogenesis, shade-avoidance response, plant immunity, and circadian clock entrainment, underlining the importance of signaling crosstalk among light, clock, hormone, and defense pathways (Yin and Ulm, 2017).

1.4 Plant Responses to Lighting Environments

1.4.1 Plant responses to light intensity, photoperiod, and daily light integral

Daily light integral (DLI) is equal to the product of photosynthetic photon flux density (PPFD, 400-700 nm) and photoperiod, representing the total photosynthetic photon flux radiated by a light source in one day, and usually has a linear relationship with plant yield. Increased DLIs were favorable for improving yield, accumulating phenolics content and essential oils in basil and perilla (*Perilla frutescens*) plants (Chang et al., 2008; Schnitzler and Habegger, 2004). However, higher DLI increases produce cost by increasing capital cost (more light fixtures) or operation cost (longer photoperiods). Therefore, a minimum target DLI for lettuce (*Lactuca sativa*) and other leafy crops in IVFs is recommended as

12-17 $mol \cdot m^{-2} \cdot d^{-1}$ (Albright et al., 2000). A few studies explored the effects of DLIs from 13.5 to 34.6 $mol \cdot m^{-2} \cdot d^{-1}$ on basil growth and development (Beaman et al., 2009; Chang et al., 2008), but no study has determined the optimum DLI between 12 and 17 $mol \cdot m^{-2} \cdot d^{-1}$ to minimize the energy cost while maintaining a high plant yield. Characterizing the response of plant growth to DLIs and the relationship between PPFD and photoperiod at same DLI are useful in lighting design and determining optimal combination of PPFD and photoperiod to obtain target DLI.

Under a controlled environment, plant growth responds almost linearly to increasing PPFD, and plant photosynthetic efficiency decreases when a light saturation point is reached. Light saturation point is specific for each plant species and for different environmental conditions. Beaman et al. (2009) reported that the lowest plant growth and edible biomass production was observed at PPFD of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and the highest at 500 and 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in basil plants grown under PPFD of 300, 400, 500, and 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ provided by FLs and incandescent lamps. Carotenoids concentration in leaf blade of four spinach (*Spinacia oleracea*) cultivars increased with increasing PPFD from 100 to 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Li et al., 2009). Similarly, there was a linear increase in both leaf fresh weight (FW) and dry weight (DW) in kale (*Brassica oleracea*) and spinach plants as PPFD increased from 125 to 620 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in a growth chamber, while the concentrations of Ca, Cu, K, and Mn in kale plants all decreased at high PPFD due to a dilution effects resulting from increased leaf FW (Lefsrud et al., 2006b). In contrast, shoot and root growth and anthocyanin content in ‘Kudo’ perilla decreased under increased

PPFD of 500 μmol·m^{-2}·s^{-1} compared to 300 μmol·m^{-2}·s^{-1}, while total polyphenol content increased (Hwang et al., 2014).

Photoperiod is the length of light in a daily cycle of 24 h. Growing plants under a low PPFD for a long photoperiod will reduce the capital costs of IVFs due to decreased number of light fixtures and requirements for cooling compared to high PPFD for a short photoperiod at same DLI. Studies showed that a long photoperiod generally increased plant biomass accumulation due to increased leaf expansion and chlorophyll content (Adams and Langton, 2005). However, many sensitive species tend to develop important physiological disorders such as leaf chlorosis and chlorophyll degradation under extended photoperiod (Kang et al., 2013; Langton et al., 2003). Sysoeva et al. (2010) reported that dry matter growth and yield of tomato (*Solanum lycopersicum*) and sweet pepper (*Capsicum annuum*) plants decreased, and light injury symptoms were observed in tomato, eggplant (*Solanum melongena*), potato (*Solanum tuberosum*), radish (*Raphanus sativus*), and cucumber (*Cucumis sativus*) plants under a 24-h photoperiod. Reduced growth and yield under extended photoperiods were thought to be caused by the inability of leaf to export accumulated photosynthates out of the leaf or the destruction of chloroplasts due to some photooxidative stress by long photoperiod (Ali et al., 2009; Demers et al., 1998).

In addition to biomass accumulation, effects of photoperiods on nutritional concentration in plants also varied. A 16-h photoperiod increased gluconasturtiin concentration by 30-40% in watercress (*Nasturtium officinale*) plants compared to a 8-h photoperiod (Engelen-Eigles et al., 2006). Similarly, lutein and β-carotene concentration

on FW basis in kale plants increased 64% and 65%, respectively, under a 6-h photoperiod compared to 24-h photoperiod, while the peak accumulation was observed in a 16-h photoperiod on DW basis (Lefsrud et al., 2006a). In contrast, betacyanin concentration in red and green amaranth (*Amaranthus tricolor*), swiss chard (*Beta vulgaris*), red beet (*Beta vulgaris*), and red spinach leaves increased from 6-h to 12-h photoperiod but decreased from 12-h to 20-h (Ali et al., 2009).

1.4.2 Plant responses to light quality

Plants sense and respond to a broad range of light spectra from UV to far-red regions, and light quality or light spectrum wavelength significantly affects plant growth, development, morphology, and secondary metabolism (Bugbee, 2016; Dou et al., 2017; Piovene et al., 2015). The development of LED technology provided researchers with opportunities to regulate plant yield and nutritional quality using different light wavelengths. However, there is no universal agreement on how light quality might affect plant yield and secondary metabolites accumulation, and plant responses to light quality are dependent on plant species, cultivars, and phytochemical compounds (Taulavuori et al., 2016).

1. Red and blue Light

Red light is sensed in plants by phytochromes and regulates responses related to seed germination, stem elongation, leaf expansion, flowering induction, etc., while blue light is sensed by cryptochromes and phototropins and regulates processes such as seedling de-etiolation, phototropism, chloroplast movement, circadian rhythms, stomatal opening, etc.

(Kozuka et al., 2005; Lobiuc et al., 2017; Neff and Van Volkenburgh, 1994). However, phytochromes, cryptochromes, and phototropins act antagonistically in the regulation of plant morphogenesis. For instance, phototropins promote leaf flattening, while phyB promotes leaf downward curling (Anpo et al., 2018). Thus, careful attention must be paid in determining the balance between blue and red light to achieve the target plant architecture.

High efficiency of red and blue lights on plant photosynthesis and growth is easily understood since they perfectly fit the absorption peak of chloroplasts (McCree, 1972). Combined red and blue (R&B) light is more effective than monochromatic red or blue light for plant growth, while monochromatic red or blue light may induce physiological disorders in several plant species (Dong et al., 2013; Sabzalian et al., 2014). For instance, monochromatic red light decreased the maximum quantum efficiency of photosystem II (F_v/F_m), stomata density, photosynthetic capacity, and impaired growth in cucumber and tomato plants and was defined as the “red light syndrome”. None of these effects occurred in leaves grown under combined R&B light (Hogewoning et al., 2010; Savvides et al., 2011; Trouwborst et al., 2016). Zheng and Van Labeke (2017) also reported that most chrysanthemum (*Chrysanthemum* × *morifolium*) cultivars had the smallest leaf area under monochromatic red light compared to blue, white, and $R_{75}B_{25}$ (combined R&B light, in which the red and blue light percentage was 75% and 25%, respectively) treatments, which indicated that a certain amount of blue light is essential to maintain normal plant growth.

Although it is generally known that combined R&B light is more effective for plant growth, the optimal red: blue (R:B) ratio or blue light proportion was not determined yet.

Shoot FW of basil plants increased by 214% when blue light proportion increased from 15% to 59% in combined R&B light at PPFD of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 16-h photoperiod (Piovene et al., 2015). Net photosynthetic rate (P_n), leaf mass per unit leaf area, and chlorophyll content per leaf area in cucumber plants increased with increasing blue light proportion from 7% to 50% (Hogewoning et al., 2010). In contrast, 'Wala' basil plants grown under white FLs with blue light proportion at 8% had higher plant height and greater shoot FW compared to plants grown under white LEDs with blue light proportion at 16% at PPFD of 160 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 16-h photoperiod, and no differences in leaf area or photosynthetic rate was observed (Fraszczak et al., 2014). Similarly, plant height, leaf area, and shoot FW and DW in cucumber plants decreased gradually with blue light proportion increased from 10% to 75% (Hernandez et al., 2016).

Numerous studies have been conducted to evaluate effects of red and blue lights on plant secondary metabolism, and results were conflicting (Cocetta et al., 2017). Several studies have shown that blue light induced the synthesis of anthocyanin and phenolic compounds in various plant species, such as lettuce, sage (*Salvia miltiorrhiza*), and gerbera (*Gerbera* hybrid) (Li, 2010; Meng et al., 2004). Accumulation of anthocyanin and phenolic compounds induced by blue light is attributed to the expression of key enzymes in the phenylpropanoid pathway, including phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), and dihydroflavonol 4-reductase (Giliberto et al., 2005; Jenkins, 2009; Son et al., 2012). In accordance, total phenolic concentration in purple basil plants and concentrations of total phenolics and anthocyanins in kale sprouts (7 days old) were

the highest under blue light treatment compared to red and white LED treatments (Hosseini et al., 2018; Qian et al., 2016). However, in contrast, rosmarinic acid concentration, the major phenolic acid in basil, was 2 times in basil plants grown under red and white LED lights compared to plants grown under blue light (Shiga et al., 2009). Hosseini et al. (2018) also reported that anthocyanin concentration in green basil plants was the highest under red light, while total phenolic concentration in green basil plants and anthocyanin concentration in purple basil plants were the highest under $R_{70}B_{30}$ treatment, compared to blue or white light treatments. One of the reasons of the contradictory results was thought to be the inconsistent light parameters among studies, such as different light sources and light intensities. Overall, the mechanism of light spectrum affecting phytochemical biosynthesis is still unclear, but it is hypothesized that red and blue lights share some mechanisms, and their effects are dependent on plant species, plant age, and the phytochemical compounds (Taulavuori et al., 2016).

2. Red and far-red light

Red and far-red lights are important signals to plants since R:FR ratio affects phytochrome regulated responses such as seed germination, seedling de-etiolation, shade avoidance, and reproduction responses (Casal, 2013; Chia and Kubota, 2010; De Wit et al., 2012). For example, low R:FR ratio decreased chlorophyll content per unit leaf area in citrus (*Citrus insitorum*), potato, white clover (*Trifolium repens*), tomato, and cucumber plants (Demotes-Mainard et al., 2016). On the contrary, EOD-FR treatment increased plant height and leaf length in tomato, arabidopsis, cucumber, and aspen (*Populus tremula*

× *tremloides*) plants compared to those grown under EOD red treatment (Chia and Kubota, 2010).

Hogewoning et al. (2012) stated that far-red light preferentially excites photosystem I (PSI), while photosynthetically active radiation (PAR, 400-700 nm) generally excite photosystem II (PSII) more than PSI, which operate in series to carry out photochemical reactions. Under relatively high level of far-red light, plants showed low quantum yield of photosynthesis since PSI tends to be over-excited relative to PSII, and vice versa (Myers, 1971; Zhen and Van Iersel, 2017). Therefore, the photosynthetic efficiency of combined far-red and PAR light should be higher than only far-red or PAR light at the same light intensity, due to a better-balanced citation of the two photosystems (Zhen and Van Iersel, 2017). Consistently, it was reported that PAR light supplemented with far-red light significantly increased quantum yield of PSII in 'Green Towers' lettuce plants and leaf area and shoot DW in geranium (*Pelargonium hortorum* 'Pinto Premium Orange Bicolor') and snapdragon (*Antirrhinum majus* 'Trailing Candy Showers Yellow') seedlings (Park and Runkle, 2017). However, some researchers hypothesized that substituting PAR light with far-red light may decrease the whole-plant photosynthetic efficiency due to a decreased PPFD. Meanwhile, far-red light substitution might increase the light radiation capture by inducing stem and petiole elongation and leaf expansion, which leave the far-red light effects on plant growth unclear (Demotes-Mainard et al., 2016; Park and Runkle, 2017).

3. Green light

It is well known that leaves absorb green (500-599 nm) light less effectively (by 16-23%) than blue or red light (Moss and Loomis, 1952). However, the average relative quantum efficiency value for broadband green light is 0.87, which is slightly lower than that for red light (0.91) and higher than that for blue light (0.73) (Sager et al., 1988). In addition, while blue and red lights are strongly absorbed by the upper level plant canopy, green light penetrates into deeper plant canopy, which could potentially increase plant yield (Terashima et al., 2009; Wang and Folta, 2013). In fact, Paradiso et al. (2011) validated that canopy quantum efficiency in 'Akito' roses (*Rosa*) grown under green light was not much lower than that grown under red light.

In addition to photosynthesis, green light also regulates plant non-photosynthetic responses such as vegetative growth, anthocyanin accumulation, and flowering initiation via phytochromes and cryptochromes (Folta and Maruhnich, 2007; Wang and Folta, 2013). Plant responses to green light share a general tendency to counteract blue or red light induced responses, such as inhibition of hypocotyl elongation (Talbott et al., 2006). For instance, as green light proportion increased, anthocyanin concentrations in arabidopsis and 'Red Sails' lettuce plants decreased significantly (Zhang and Folta, 2012; Zhang et al., 2011). Stomatal opening stimulated by blue light could also be reversed by green light in a range of plant species and supplemental green light to red and blue LEDs induced shade avoidance responses in arabidopsis plants (Frechilla et al., 2000; Talbott et al., 2002; Zhang and Folta, 2012).

4. UV-B light

UV-B light is commonly considered as a stress factor to plant growth due to its excess excitation energy unavoidably leading to the production of reactive oxygen species in plant organelles, such as chloroplasts, mitochondria, and peroxisomes. Recently, some studies indicated that supplemental UV-B radiation induced secondary metabolite synthesis in plants, such as anthocyanins, flavonoids, ascorbate, carotenoids, glutathione and a broad range of other metabolites, which provide plant protection against potential UV-B damage and health benefits in human diets (Ghasemzadeh et al., 2016; Sakalauskaite et al., 2013). Supplemental UV-B radiation at 2.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 1 h or 2 h per day significantly increased the content of total phenolic compounds, anthocyanin concentrations, and antioxidant activity in basil plants without suppressing biomass accumulation, and 1 h UV-B treatment was more efficient for anthocyanin accumulation than 2 h treatment (Sakalauskaite et al., 2012; Sakalauskaite et al., 2013). Similarly, supplemental UV-A radiation to white LEDs enhanced antioxidant content in 'Genovese' basil microgreens (Brazaityte et al., 2016). However, high levels of UV-B radiation generally damages photosynthetic apparatus, depresses plant growth, and reduces plant yield (Wargent and Jordan, 2013; Wargent et al., 2009). Therefore, further research is needed to find the balance between enhanced nutritional quality and yield reduction.

1.5 Objectives

There are increasing interests to produce culinary herbs and leafy greens in IVFs due to increasing population and urbanization, resource competition, and climate change.

Among artificial light sources, LEDs have several advantages such as wavelength specificity, high energy conversion efficiency, low heat emission, and long lifespan which attracted attention as the preferred source of artificial lights for crop production in IVFs. To further improve plant productivity and quality in IVFs with greater energy saving and sustainability, objectives of the present study were (i) to characterize the minimum light requirements (DLI) for the production of culinary herbs and leafy greens in IVFs without significant decrease in plant yield or nutritional value; (ii) to investigate the effects of different light quality including red, blue, and green lights (white fluorescent light and white LED lights) and combined R&B LEDs with different blue light proportions on plant photosynthesis, morphology, yield, and phytochemical accumulation; (iii) to determine the optimal dose of UV-B radiation to achieve enhanced accumulation of secondary metabolites in leaf herbs and vegetables without significant yield reduction; and (iv) to evaluate the effects of far-red and green lights on plant growth, yield, and nutritional quality.

CHAPTER II

RESPONSES OF BASIL PLANTS TO DIFFERENT DAILY LIGHT INTEGRALS IN PHOTOSYNTHESIS, MORPHOLOGY, YIELD, AND NUTRITIONAL QUALITY*

2.1 Synopsis

Consumption of basil (*Ocimum basilicum*) plants has been increasing worldwide in recent years due to various health benefits it offers. To achieve a stable supply of basil products of high nutritional quality, more growers are turning to controlled environment production with artificial lighting (indoor vertical farms, IVFs) due to its high environmental controllability and sustainability. However, electricity cost for lighting is a major limiting factor to the commercial application of IVFs, and little information is available on the minimum light requirement to produce uniform and high-quality basil products. To determine the optimal daily light integral (DLI) for basil production in IVFs, this study investigated the effects of five DLIs, 9.3, 11.5, 12.9, 16.5, and 17.8 $mol \cdot m^{-2} \cdot d^{-1}$, on basil growth and quality. 'Genovese' basil plants were treated with five DLIs provided by white fluorescent lamps for 21 days after germination, and gas exchange rate, growth, yield, and nutritional quality of basil plants were measured to evaluate the effects of different DLIs on basil growth and quality. Results indicated that basil plants grown under higher DLIs of 12.9, 16.5 or 17.8 $mol \cdot m^{-2} \cdot d^{-1}$ showed improved photosynthesis, compared to those under lower DLIs of 9.3 and 11.5 $mol \cdot m^{-2} \cdot d^{-1}$. High DLIs resulted in lower Chl a+b content per leaf fresh weight, higher Chl a/b ratios, and larger and thicker

*Reprinted with permission from Dou, H., G. Niu, M. Gu, and J.G. Masabni. 2018. Responses of sweet basil to different daily light integrals in photosynthesis, morphology, yield, and nutritional quality. HortScience 53:496-503. Copyright 2018 by the American Society for Horticultural Science.

leaves of basil plants. Shoot fresh weight under DLIs of 12.9, 16.5 and 17.8 $mol \cdot m^{-2} \cdot d^{-1}$ was 54.2%, 78.6%, and 77.9%, respectively, higher than that at DLI of 9.3 $mol \cdot m^{-2} \cdot d^{-1}$. Additionally, higher DLIs led to higher soluble sugar content and dry matter ratio compared to lower DLIs. Contents of anthocyanin, phenolics, and flavonoids of basil leaves were also positively correlated to DLIs, and antioxidant capacity at DLI of 17.8 $mol \cdot m^{-2} \cdot d^{-1}$ was 73% higher than that at DLI of 9.3 $mol \cdot m^{-2} \cdot d^{-1}$. Combining results of growth, yield, and nutritional quality of basil plants, we suggest a DLI of 12.9 $mol \cdot m^{-2} \cdot d^{-1}$ for basil commercial production in IVFs to minimize the energy cost while maintaining a high yield and nutritional quality.

2.2 Introduction

Basil plants are often referred as the “king of herbs” or the “royal herb”, and is widely used in cooking and medicinal practices, as well as a fragrant, ornamental plant for gardens and containers because of its unique flavor and relatively high content of essential oils and phenolic compounds (Chiang et al., 2005; Kruma et al., 2008; Makri and Kintzios, 2008). The United States is both the largest producer and importer of basil plants in the world, with most of its production in open fields (DAFF, 2012). However, the yield and quality such as nutritional contents of basil plants grown outdoors is hard to control and its phytochemical concentration varies widely with cultivation location, season, and cultivar (Fischer et al., 2011; Hassanpouraghdam et al., 2010; Pushpangadan and George, 2012). To achieve a stable and reliable supply of basil plants, more growers are adopting indoor controlled environment production, which has proven to be a suitable alternative to open field and greenhouse production (Liaros et al., 2016; Saha et al., 2016).

Indoor vertical farms (IVF), also known as “plant factory”, is a highly controlled environmental system for plant production that utilizes multiple-layer culture shelves with artificial lighting (Despommier, 2010; Kozai et al., 2015). In consideration of global climate change and increasing urban populations, food security is an increasingly pressing matter, especially considering limited resources such as arable land, clean water, and fuel energy (Dunwoody, 2014; Liaros et al., 2016). Indoor vertical farming emerged as an environmentally sustainable plant production system due to its high resource-use efficiency of both land and water (Despommier, 2013; Kozai, 2013; Kozai et al., 2015; Touliatos et al., 2016). The utilization efficiency of land, water, CO_2, and light energy in indoor vertical farming were 100, 40, 2, and 1.7 times of those in greenhouses, respectively (Kozai, 2007; Ohyama et al., 2003; Yokoi et al., 2005). In recent years, the number of IVFs has increased rapidly in Japan, China, and the other Asian countries (Kozai et al., 2015). In North America, IVFs have been built for commercial production of leafy greens, herbs, and transplants (Kozai et al., 2015). For example, AeroFarms, an enterprise specializing in indoor farming, built its ninth farm in Newark, New Jersey, and is the world’s largest indoor vertical farm based on annual output (AeroFarms, 2017). As one of the most popular herbs in the United States, basil is a great candidate plant for IVFs due to its high value and demand (Liaros et al., 2016), and basil plants are adapted to moderately high light intensity and long day irradiation (Pushpangadan and George, 2012).

Light is one of the most important environmental factors that affects plant development and regulates plant behavior depending on light quantity, quality, direction, and duration

(Chang et al., 2008; Dou et al., 2017; Figueiredo et al., 2008; Shafiee-Hajiabad et al., 2016). Daily light integral (DLI) represents the total photosynthetic photon flux radiated by a light source in 24 h, and usually has a linear relationship with plant yield and nutrient accumulation (Bochenek and Fallstrom, 2016; Colonna et al., 2016; Dai et al., 2009). In IVFs, powering artificial lighting is one of the most electricity consumption factors, which makes energy conservation one of the biggest concerns for its commercial adoption (Ohyama et al., 2002). DLIs of 12-17 $mol \cdot m^{-2} \cdot d^{-1}$ are recommended for vegetables and herbs in IVFs in terms of energy savings (Albright et al., 2000; Kozai et al., 2015). A few studies explored the effects of DLIs from 13.5 to 34.6 $mol \cdot m^{-2} \cdot d^{-1}$ on basil growth and development (Beaman et al., 2009; Chang et al., 2008), but no study has determined the optimum DLI between 12 and 17 $mol \cdot m^{-2} \cdot d^{-1}$ for basil production under an indoor controlled environment. Between DLIs of 17.3 and 23.0 $mol \cdot m^{-2} \cdot d^{-1}$, no differences in plant height, canopy diameter, or shoot yield among 'Genovese', 'Italian Large Leaf', and 'Nufar' basil were observed, which were lower than basil grown under DLIs of 28.8 and 34.6 $mol \cdot m^{-2} \cdot d^{-1}$ in a growth chamber, respectively (Beaman et al., 2009). In a glasshouse condition, there was no difference in photosynthesis of 'Genovese' basil between DLI of 13.5 $mol \cdot m^{-2} \cdot d^{-1}$ (light shading in a glasshouse) and 24.9 $mol \cdot m^{-2} \cdot d^{-1}$ (full sunlight), while DLI of 5.3 $mol \cdot m^{-2} \cdot d^{-1}$ (heavy shading) significantly reduced the photosynthetic rate, leaf area, shoot fresh weight (FW), and total essential oils content (Chang et al., 2008). The total amount of essential oil of 'Bageco' basil increased significantly with supplemental light provided by high pressure sodium-vapor lamp compared to plants grown under sunlight (Nitz and Schnitzler, 2004). Based on these circumstances, the objective of this

study was to determine the minimum DLI for basil production with comparable nutritional values in IVFs.

2.3 Materials and Methods

2.3.1 Plant materials and growing conditions

The experiment was conducted in a walk-in growth room in Texas AgriLife Research and Extension Center at El Paso, TX from 7 March to 26 April 2017 and repeated from 17 April to 29 May. 'Improved Genovese Compact' green basil (Johnny's Selected Seeds, Winslow, ME, USA) was used in both experiments. For both experiments, one basil seed per cell was sown in 72 square cell trays (length 3.86 cm; height 5.72 cm; volume 59 cm^3) with Metro-Mix 360 (peat moss 41%, vermiculite 34%, pine bark 25%, Sun Gro® Horticulture, Bellevue, WA, USA). All trays were placed under mist in a greenhouse for germination. Seedlings were moved out from mist after germination and grown in a greenhouse for two weeks. Seedlings were then transplanted to 4" square pots (length 9.52 cm, height 8.26 cm; volume 574 cm^3) with Metro-Mix 360, when roots were visible on the outside of the plug root ball. Uniform plants were selected and moved to the walk-in growth room for different DLI treatments for 21 days.

2.3.2 DLIs treatments

There were five DLI levels, 9.3, 11.5, 12.9, 16.5, and 17.8 $mol \cdot m^{-2} \cdot d^{-1}$ (hereafter, DLI 9.3, DLI 11.5, DLI 12.9, DLI 16.5, and DLI 17.8), created by growing basil plants under five different light intensities of 160, 200, 230, 290, or 310 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively,

with the same 16-h photoperiod provided by Cool White Alto Linear Fluorescent Lamps (FLs, Philips Lighting, Somerset, NJ, USA). All treatments were randomly arranged in the growth room, and 18 plants were randomly planted in each treatment (replications). To minimize light distribution being disproportionate within each treatment, all plants were systematically rearranged every three days. The light intensity in each treatment was measured at 15 cm from FLs at 9 spots using PS-100 spectroradiometer (Apogee Instruments, Logan, UT, USA). All plants were sub-irrigated with nutrient solution containing 1.85 $g \cdot L^{-1}$ (277.5 ppm N) 15N-2.2P-12.5K (Peters 15-5-15 Ca-Mg Special, The Scotts Company, Marysville, OH, USA) as needed, maintaining electrical conductivity of 2.0 $dS \cdot m^{-1}$ and pH of 6.0. Plant canopy temperature was recorded and maintained at 24.5/21.3ºC day/night. Mechanical mini fans (LS1225A-X, AC Infinity, City of Industry, CA, USA) were used to circulate the air to achieve uniform temperatures across treatments. Both experiments showed a similar trend, thus only data from the second experiment are presented.

2.3.3 Measurements

1. Gas exchange and chlorophyll concentration analysis

A portable gas exchange analyzer (CIRAS-3, PP Systems International, Amesbury, MA, USA) was used to measure the gas exchange rate of basil leaves on D20. A PLC3 leaf cuvette with LED light unit was used, and light intensity, relative air humidity, and CO_2 concentration inside the leaf chamber were kept constant at 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 50%, and 390 $\mu mol \cdot mol^{-1}$, respectively. The soil plant analysis development (SPAD) index of

basil plants was recorded weekly to quantify relative chlorophyll (Chl) content in basil leaves using a Chl meter SPAD-502 (Konica-Minolta cooperation, Ltd., Osaka, Japan). On D21, approximately 0.2 g of basil leaves were cut into small pieces, then extracted in 80% methanol (v:v) for three days. The absorbance of extracts was measured at 663 nm and 645 nm using a spectrophotometer (Genesys 10S UV/Vis, Thermo Fisher Scientific, Madison, WI, USA), and the concentrations of Chl a and Chl b were calculated according to Porra et al. (1989). The Chl a+b and Chl a/b were calculated accordingly.

2. Growth parameters

Growth characteristics such as plant height, two perpendicular widths, and the number of internodes were recorded on day 1 (D1) of the treatment and then weekly. Six plants per treatment were randomly selected for measurement. Height and two perpendicular widths of the first branch of basil plants were measured on D21, the end of the experiment. Leaf area was measured using a leaf area meter (LI-3100, LI-COR, Lincoln, NE, USA), and shoot and root FW were recorded on D21. The shoot and root tissues were dried at 80ºC in a drying oven (Grieve, Round Lake, IL, USA) for 3 days to determine dry weight (DW).

3. Nutritional quality measurement

Six plants per treatment were randomly selected for measurements of soluble sugar content, anthocyanin concentration, total phenolic concentration, total flavonoid concentration, and antioxidant capacity of basil leaves on D21 to evaluate the effects of DLIs on basil nutritional quality. The soluble sugar content of fresh basil leaves was

measured using a Brix Refractometer (Extech Instruments, Nashua, NH, USA). Fresh leaves were collected in a cooler and immediately stored in a deep freezer (IU1786A, Thermo Fisher Scientific, Marietta, OH, USA) at -80ºC until phytochemical analyses.

Extraction. Approximately 2 g fresh basil leaves were ground in liquid nitrogen and extracted with 15 mL 1% acidified methanol in darkness. After overnight extraction, the mixture was centrifuged (Sorvall RC 6 Plus Centrifuge, Thermo Fisher Scientific, Madison, WI, USA) at 13,200 rpm (26,669 ×*g*) for 15 min, and the supernatant was collected for phytochemical analysis.

Anthocyanin analysis. The absorbance of extracts was measured at 530 nm using the aforementioned spectrophotometer, and the anthocyanin concentration was expressed as mg cyanidin-3-glucoside equivalents using a molar extinction coefficient of 29,600 (Connor et al., 2002). Since the extracts were freshly prepared from leaf tissues maintained at -80ºC and did not undergo extensive processing or significant browning, a pH differential method for anthocyanin content was considered unnecessary (Connor et al., 2002).

Phenolics analysis. The total phenolic concentration of basil leaves was determined using the modified Folin-Ciocalteu reagent method (Xu and Mou, 2016) described as the following: 100 μL extraction sample was added to a mixture of 150 μL distilled water and 750 μL 1/10 dilution Folin-Ciocalteu reagent. After 6 min reaction, 600 μL 7.5% Na_2CO_3 was added to the mixture. The mixture was incubated at 45°C in a water bath for 10 min before the absorbance was measured at 725 nm using a microplate reader (ELx800,

BioTek, Winooski, VT, USA). Results were expressed as mg gallic acid equivalent g^{-1} FW of basil leaves.

Flavonoids analysis. The total flavonoid concentration of basil leaves was determined as the following (Xu and Mou, 2016): 20 μL extraction sample was added to a mixture of 85 μL distilled water and 5 μL 5% $NaNO_2$. After 6 min, 10 μL of 10% $AlCl_3 \cdot 6H_2O$ was added. After 5 min, 35 μL of 1M NaOH and 20 μL distilled water was added, then the absorbance was measured at 520 nm using the aforementioned microplate reader. The results were expressed as mg of (+)-catechin hydrate equivalent per unit FW of basil leaves. The content of total anthocyanin, phenolic compound, and flavonoid per basil plant were calculated by multiplying the content of anthocyanin, phenolic compound, and flavonoid by leaf FW per plant.

Antioxidant capacity analysis. The total antioxidant capacity of basil leaves was measured using the ferrous ion chelating activity method (Xu and Mou, 2016) described as the following: the mixture of 24 μL extracts, 1.20 mL methanol, and 16 μL of 2 mM ferrous chloride were vortexed vigorously. A 32 μL of 5 mM ferrozine was then added and mixed vigorously, and the absorbance of mixture was measured at 562 nm after 4 min reaction using the aforementioned spectrophotometer. Ferrous ion chelating activity was calculated as the absorbance difference between control and sample.

2.3.4 Statistical analysis

One-way analysis of variance (ANOVA) was conducted to analyze the effects of DLI on all measured parameters. Mean comparison among treatments was conducted using

Student's t method. Correlation test was conducted using Pairwise Correlations method. All statistical analyses were performed using JMP (Version 13, SAS Institute Inc., Cary, NC, USA).

2.4 Results

2.4.1 Photosynthesis and chlorophyll content of basil leaves under different DLIs

Relative Chl content of basil leaves, SPAD readings, increased significantly as basil growth stage developed and DLI increased (Fig. 1A). SPAD for treatments DLI 9.3, DLI 11.5, and DLI 12.9 increased from 30 to 37 after 21 days treatment, while those in the DLI 16.5 and DLI 17.8 treatments increased to approximately 41, which was 11% higher (Fig. 1A). In contrast, no difference in Chl a concentration per leaf FW was observed among the five different DLIs on D21, while Chl b content was higher for treatments DLI 9.3 and DLI 11.5, and lower for treatments DLI 12.9, DLI 16.5, and DLI 17.8 (Fig. 1B). Higher levels of Chl a/b ratio (Fig. 1C) and lower levels of Chl a+b content (Fig. 1B) were observed for treatments DLI 12.9, DLI 16.5, and DLI 17.8. Chl a+b content per leaf FW for treatments DLI 9.3 and DLI 11.5 were about 17% higher than basil plants grown under treatments DLI 12.9, DLI 16.5, and DLI 17.8 (Fig. 1B).

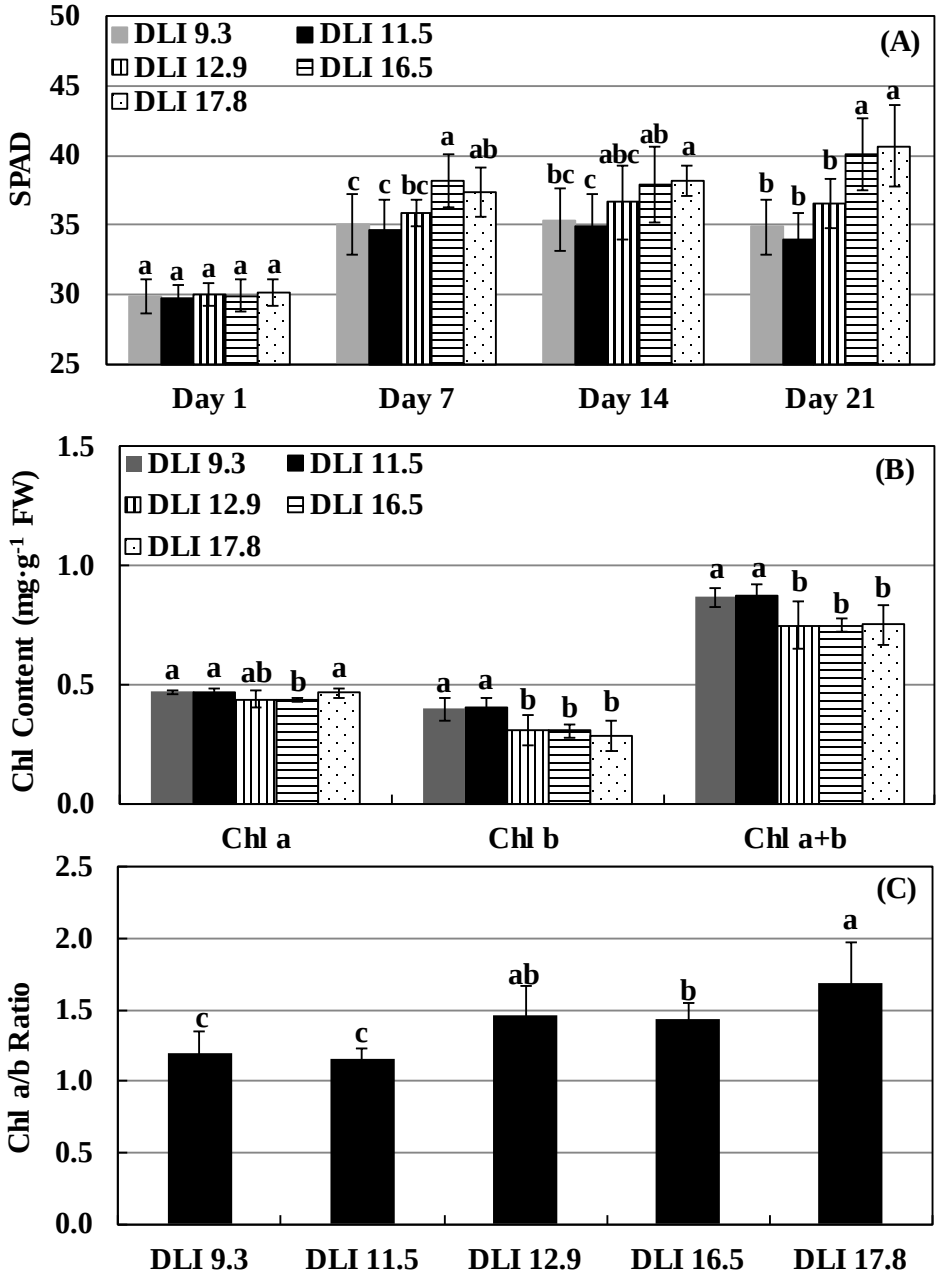

Figure 1. Relative Chl content of basil leaves from day 1 to day 21 (A), Chl a, Chl b, and Chl a+b content (B), and Chl a/b ratio (C) of 'Improved Genovese Compact' basil plants grown for 21 days at different daily light integrals (DLI). Means followed by the same lowercase letters are not significantly different, according to Student's t mean comparison ($P < 0.05$). Bars represent standard errors. Reprinted with permission from Dou et al. (2018).

Leaf net photosynthetic rate per leaf area (P_n), transpiration (E), and stomatal conductance (G_s) of basil leaves increased significantly as DLI increased, and were the highest for treatments DLI 12.9, DLI 16.5, and DLI 17.8 (11.5, 10.6, and 10.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$), followed by treatments DLI 9.3 and DLI 11.5 (6.1 and 7.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$), respectively (Table 2). Net photosynthetic rate for treatments DLI 12.9 was 86% and 47% higher than treatments DLI 9.3 and DLI 11.5, respectively, and no difference was observed among treatments DLI 12.9, DLI 16.5, or DLI 17.8 (Table 2). Transpiration for treatment DLI 12.9 was 78% and 57% higher than treatments DLI 9.3 and DLI 11.5, respectively, while G_s for treatments DLI 12.9 was 126% and 83% higher (Table 2).

Table 2. Net photosynthetic rate per leaf area (P_n), transpiration (E), stomatal CO_2 concentration (C_i), and stomatal conductance (G_s) of 'Improved Genovese Compact' basil leaves grown for 20 days at different daily light integrals (DLIs). A portable gas exchange analyzer CIRAS-3 was used to measure the gas exchange rate of basil leaves at harvest. Adapted with permission from Dou et al. (2018).

Treatment	P_n ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	E ($mmol \cdot m^{-2} \cdot s^{-1}$)	C_i ($\mu mol \cdot mol^{-1}$)	G_s ($mmol \cdot m^{-2} \cdot s^{-1}$)
DLI 9.3	6.1 c[z]	1.26 c	266 a	86 b
DLI 11.5	7.8 bc	1.43 bc	255 a	106 b
DLI 12.9	11.5 a	2.24 a	269 a	194 a
DLI 16.5	10.6 a	2.01 a	273 a	172 a
DLI 17.8	10.4 ab	1.85 ab	252 a	142 ab

[z] Means followed by the same lowercase letters are not significantly different, according to Student's t mean comparison ($P < 0.05$).

2.4.2 Morphological differences of basil plants influenced by DLIs

Basil plants grown under higher DLIs had a larger canopy due to increased height and width (Table 3) but had similar number of internodes (data not presented). Plant width

responded faster to DLIs compared to plant height, with visible difference after one week DLI treatment, whereas it took two weeks for plant height to show difference among treatments. On D21, plant height was the greatest for treatments DLI 12.9, DLI 16.5, and DLI 17.8 (22.1, 23.3, and 23.0 cm, respectively), followed by DLI 11.5 (20.2 cm), and was the lowest for DLI 9.3 (17.4 cm). Although plant width showed visual differences earlier than plant height, the differences among five DLI treatments were small (Table 3).

Table 3. Plant height and width of 'Improved Genovese Compact' basil plants on 1, 7, 14, and 21 days after transplanting at different daily light integrals (DLIs). Adapted with permission from Dou et al. (2018).

Treatment	Day 1		Day 7		Day 14		Day 21	
	Height (cm)	Width (cm)	Height (cm)	Width (cm)	Height (cm)	Width (cm)	Height (cm)	Width (cm)
DLI 9.3	3.9 a[z]	5.1 a	5.3 a	7.7 b	10.1 b	10.2 b	17.4 c	12.5 b
DLI 11.5	3.8 a	5.4 a	5.5 a	7.8 b	12.1 a	10.5 ab	20.2 b	13.0 ab
DLI 12.9	4.0 a	5.2 a	6.1 a	8.3 ab	12.7 a	10.9 a	22.1 a	12.8 ab
DLI 16.5	3.7 a	4.9 a	6.0 a	8.1 ab	12.9 a	11.0 a	23.3 a	13.0 ab
DLI 17.8	3.8 a	5.1 a	6.3 a	8.6 a	13.0 a	10.8 a	23.0 a	13.4 a

[z] Means followed by the same lowercase letters are not significantly different, according to Student's t mean comparison ($P < 0.05$).

Basil plants grown under higher DLIs had larger and thicker leaves, as well as greater branch height and width (Table 4). With similar number of leaves, total leaf area for treatment DLI 17.8 was 51% and 35% higher than treatments DLI 9.3 and DLI 11.5, respectively, while specific leaf area (leaf area per unit leaf DW) was 30% and 21% lower. Lower specific leaf area under higher DLIs indicated that the thickness of basil leaves increased as DLIs increased. In addition to plant height and width, branching of basil plants was also positively correlated to DLIs. There were two pairs of fully expanded

leaves at the 1st branch of basil plants grown under treatments DLI 12.9, DLI 16.5, and DLI 17.8 while only one pair of fully expanded leaves for treatment DLI 9.3 (data not presented), which contributed to increased branch height and width under higher DLIs (Table 4).

Table 4. Leaf area, specific leaf area, and 1st branch height and width of 'Improved Genovese Compact' basil plants grown for 21 days at different daily light integrals (DLIs). Adapted with permission from Dou et al. (2018).

Treatment	Leaf area (cm^2)	Specific leaf area[z] ($cm^2 \cdot g^{-1}$, DW)	Height of 1st branch (cm)	Width of 1st branch (cm)
DLI 9.3	406 b[y]	518 a	2.9 c	3.8 b
DLI 11.5	454 b	480 ab	4.5 b	5.0 a
DLI 12.9	560 a	462 b	5.4 ab	5.7 a
DLI 16.5	609 a	389 c	6.2 a	5.7 a
DLI 17.8	614 a	398 c	6.3 a	5.9 a

[z] Specific leaf area = leaf area per unit leaf dry weight.
[y] Means followed by the same lowercase letters are not significantly different, according to Student's t mean comparison ($P < 0.05$).

2.4.3 Plant growth and yield of basil plants under different DLIs

The highest shoot FW was observed in treatments DLI 12.9, DLI 16.5, and DLI 17.8 (20.2, 23.4, and 23.3 g, respectively), followed by DLI 11.5 (15.7 g), while DLI 9.3 (13.1 g) had the lowest value (Fig. 2A). Fresh leaf and stem weight had the similar trend as fresh shoot yield, while root FW was the highest in treatments DLI 16.5 and DLI 17.8, followed by DLI 12.9, then DLI 11.5, and was the lowest in DLI 9.3. Leaf DW was more sensitive to DLIs compared to leaf FW, and significant differences were observed among treatments DLI 12.9, DLI 16.5, and DLI 17.8 (1.22, 1.58 and 1.55g, respectively) (Fig. 2B). Shoot DW had a similar pattern with leaf DW, where shoot DW in DLI 17.8 was over twofold

that in DLI 9.3. Shoot FW and DW of basil plants were both positively correlated to DLIs at the time of harvest on D21 (Fig. 3A). Shoot dry matter content of basil plants was also positively influenced by DLIs, ranging from 6.7% to 9.2% (Fig. 3B).

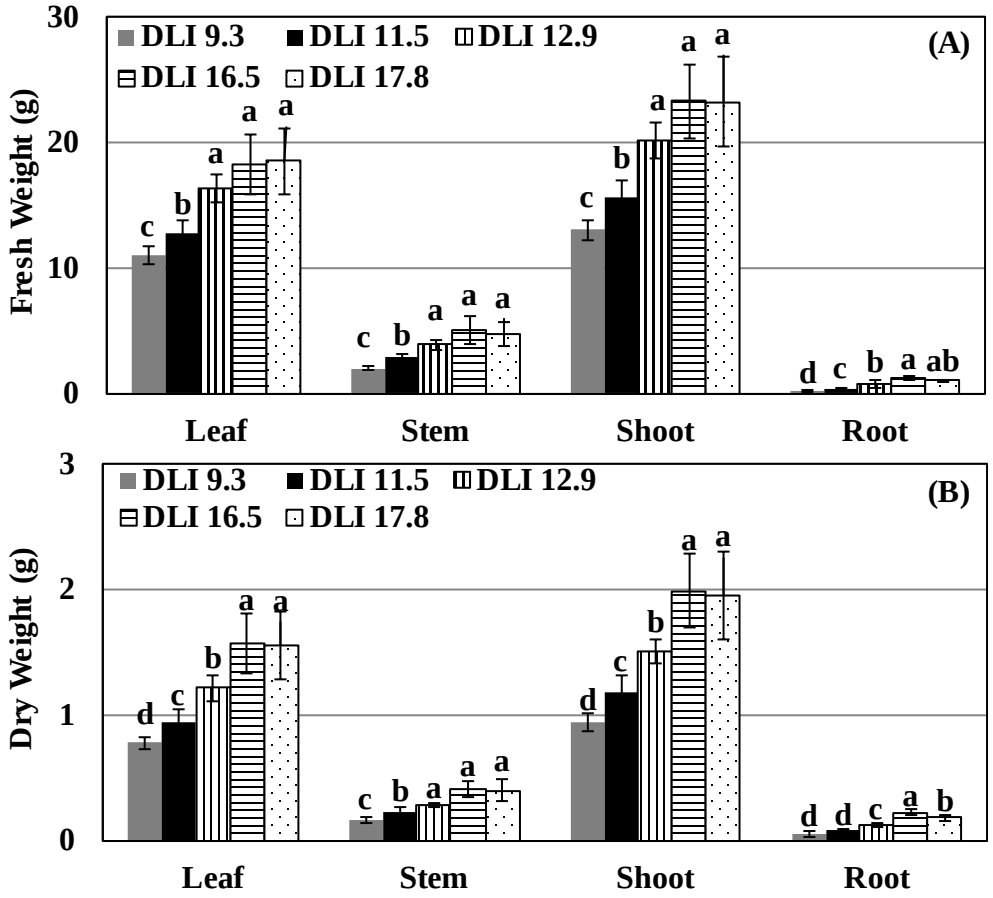

Figure 2. Leaf, stem, shoot, and root fresh weight (A), and dry weight (B) of 'Improved Genovese Compact' basil plants grown for 21 days at different daily light integrals (DLIs). Means followed by the same lowercase letters are not significantly different, according to Student's t mean comparison ($P < 0.05$). Bars represent standard errors. Adapted with permission from Dou et al. (2018).

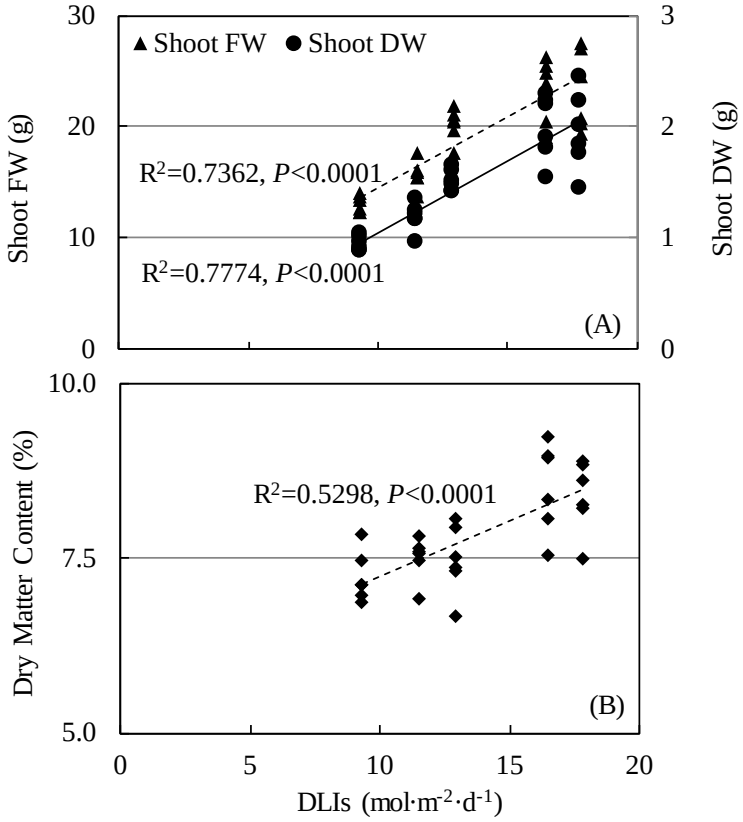

Figure 3. Correlations between daily light integrals (DLIs) and shoot fresh weight (FW) and dry weight (DW) (A), and correlations between DLIs and dry matter content (B) in 'Improved Genovese Compact' basil plants grown for 21 days at different DLIs. Dash lines show regression between measured parameters and DLIs according to Pairwise Correlation method. Adapted with permission from Dou et al. (2018).

2.4.4 Nutritional quality of basil leaves under different DLIs

Soluble sugar content, total phenolic concentration, and total flavonoid concentration of basil leaves increased with DLIs, and were 52%, 35%, and 85% higher in treatment DLI 17.8 compared to DLI 9.3, respectively (Table 5). There was no difference in anthocyanin concentration among different DLIs, ranging from 2.60 to 2.82 $mg \cdot 100g^{-1}$ leaf FW (Table 5). Increased phenolic compound and flavonoid concentration of basil

leaves led to higher antioxidant capacities with increasing DLIs, which was 73% higher in treatment DLI 17.8 than DLI 9.3 (Table 5). Owing to higher leaf FW under higher DLIs, total anthocyanin content, phenolic content, and flavonoid content per plant were positively correlated to DLIs (Fig. 4).

Table 5. Brix, anthocyanin concentration, phenolics concentration, flavonoids concentration, and antioxidant capacity of 'Improved Genovese Compact' basil leaves grown for 21 days at different daily light integrals (DLIs). Adapted with permission from Dou et al. (2018).

Treatment	Brix (%)	Anthocyanin concentration ($mg \cdot 100g^{-1}$)	Phenolics concentration ($mg \cdot g^{-1}$)	Flavonoids concentration ($mg \cdot g^{-1}$)	Antioxidant capacity (%)
DLI 9.3	2.3 c[z]	2.60 a	1.02 b	0.34 c	1.96 b
DLI 11.5	2.7 bc	2.76 a	1.07 b	0.47 b	3.46 ab
DLI 12.9	2.9 b	2.82 a	0.99 b	0.40 bc	3.80 ab
DLI 16.5	2.5 bc	2.82 a	1.61 a	0.90 a	5.26 a
DLI 17.8	3.5 a	2.73 a	1.38 a	0.63 a	3.37 ab

[z] Means followed by the same lowercase letters are not significantly different, according to Student's t mean comparison ($P < 0.05$).

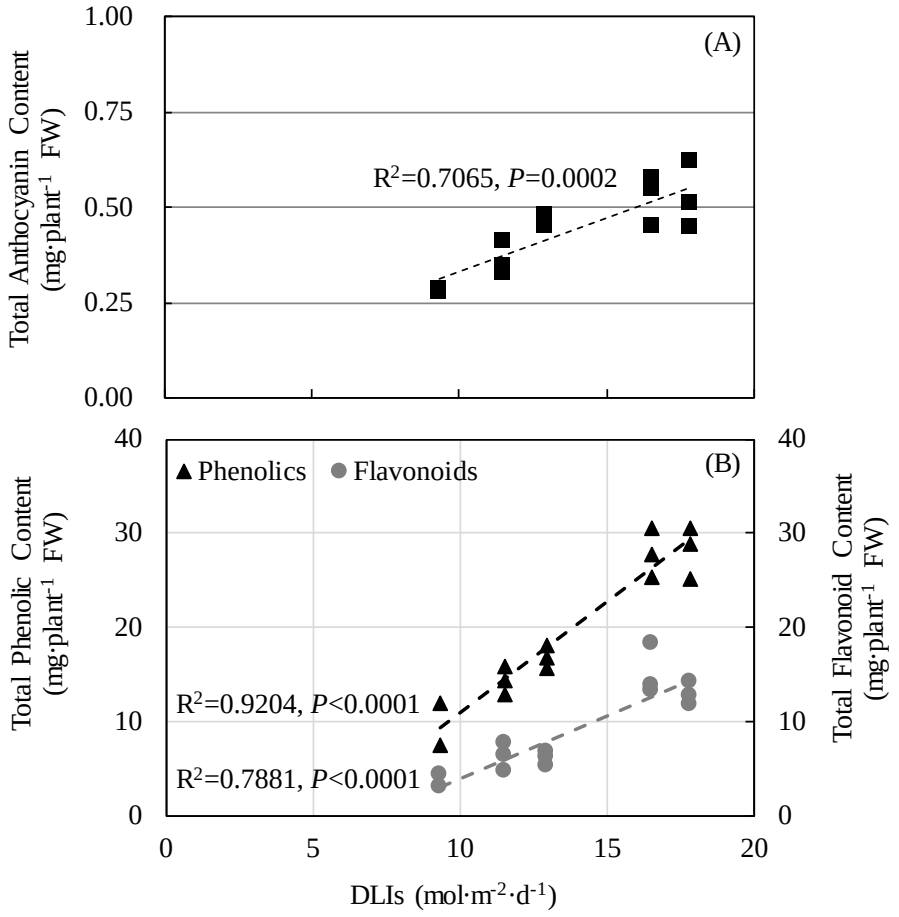

Figure 4. Correlations between daily light integrals (DLIs) and total anthocyanin content per plant (A), and correlations between DLIs and total phenolic content (gallic acid equivalent) and total flavonoid content ((+)-catechin hydrate equivalent) per plant (B) in 'Improved Genovese Compact' basil plants grown for 21 days at different DLIs. Dash lines show regression between measured parameters and DLIs according to Pairwise Correlation method. Adapted with permission from Dou et al. (2018).

2.5 Discussion

2.5.1 Photosynthetic capacity, Chl content, leaf morphology, growth, and yield of basil plants

As a significant factor affecting plant photosynthesis, DLI or light intensity alters leaf Chl content to maximize photosynthetic efficiency and productivity (Retkute et al., 2015; Wittmann et al., 2001). In this study, P_n of basil leaves increased from 6.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in treatment DLI 9.3 (relatively low light intensity of 160 $\mu mol \cdot m^{-2} \cdot s^{-1}$) to 10.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in treatment DLI 17.8 (relatively high light intensity of 310 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (Table 2), indicating that the light saturation point of basil is higher than 310 $\mu mol \cdot m^{-2} \cdot s^{-1}$ under this environment. Similarly, Polyakova et al. (2015) reported that P_n of 'Ararat' basil leaves grown for 30 days under 240-260 $\mu mol \cdot m^{-2} \cdot s^{-1}$ provided by induction lamps was over twice higher than plants under 80-85 $\mu mol \cdot m^{-2} \cdot s^{-1}$ provided by white LEDs. One reason for the increased P_n of high-light leaves is their generally higher Chl content per leaf area (Lichtenthaler et al., 2007). Net photosynthetic rate represents the sum of individual cell CO_2 assimilation per leaf area, and thinner leaves under lower DLIs contain significantly less cells per leaf area as compared to thicker leaves under higher DLIs (Table 4), consequently resulting in lower Chl content per leaf area (SPAD) and P_n (Fig. 1A and Table 2). SPAD reading of plants was mainly associated with a greater amount of nitrogen per leaf area, as well as higher content of Rubisco enzyme, and subsequently resulted in increased photosynthesis (Lichtenthaler, 1985). Increased SPAD reading also led to darker green leaves of basil plants under higher DLIs, which plays an important role for

consumers making purchasing decisions (Rouphael et al., 2012). Basil plants under higher DLIs exhibited higher P_n not only on leaf area basis but also on Chl basis and leaf DW basis (Fig. 5), which could be explained by the possession of chloroplasts adapted to higher light intensity under higher DLIs. High-light adapted chloroplasts had higher photosynthetic quantum conversion rate with adapted ultrastructure, biochemical organization and a special arrangement of chlorophylls and carotenoids in the thylakoids under higher DLIs, resulting in increased P_n on Chl basis and leaf DW basis (Lichtenthaler et al., 2007).

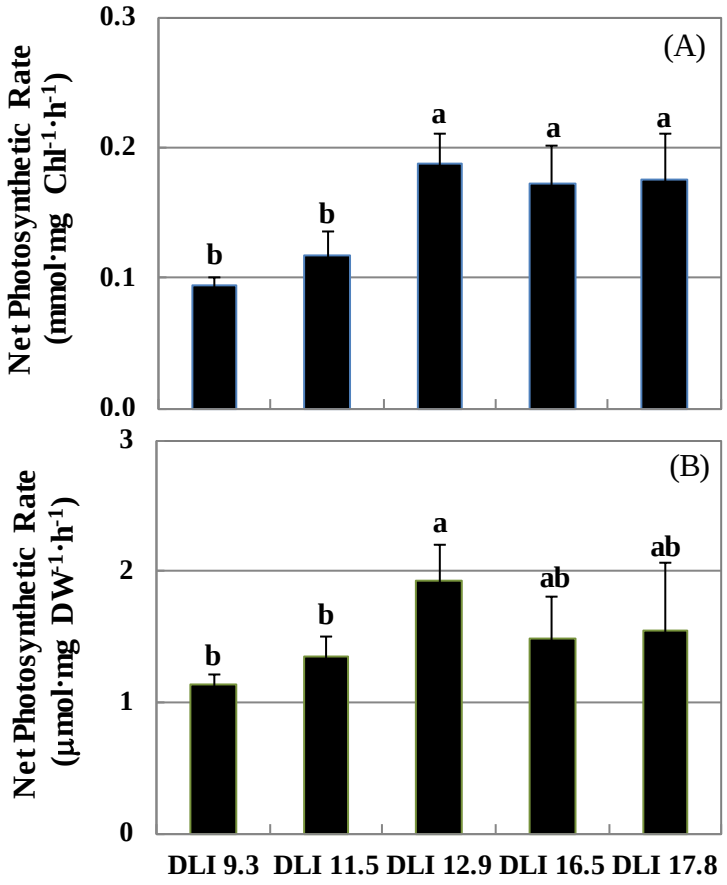

Figure 5. Net photosynthetic rate per Chl content (A) and per leaf DW (B) of 'Improved Genovese Compact' basil plants grown for 21 days at different daily light integrals (DLIs). Means followed by the same lowercase letters are not significantly different, according to Student's t mean comparison (P < 0.05). Bars represent standard errors. Adapted with permission from Dou et al. (2018).

In contrast to Chl content on leaf area basis, basil leaves under lower DLIs had a significantly higher Chl a+b content per leaf FW, and treatment DLI 9.3 was up to 16% higher than treatment DLI 17.8 (Fig. 1B). This result was consistent with the Chl a+b content of 'Ararat' basil and Chinese liquorice (*Glycyrrhiza uralensis*) plants grown under different DLIs (Hou et al., 2010; Polyakova et al., 2015). Increased Chl a+b levels of basil

leaves under lower DLIs resulted from increased Chl b levels with similar Chl a content, and consequently lower Chl a/b ratios (Fig. 1C). The difference in Chl a/b ratios is also a useful indicator of light conditions, with lower Chl a/b ratios in shade leaves and higher Chl a/b ratios in sun leaves (Sarijeva et al., 2007). Under lower DLIs, plants maximize light-harvesting capacity by increasing light harvesting chlorophyll-protein complex in photosystem II, which contains mainly of Chl b, and consequently a higher Chl b content and lower Chl a/b ratio (Kitajima and Hogan, 2003; Sarijeva et al., 2007). The increased Chl a+b content per leaf FW under lower DLIs demonstrated the plants' ability to maximize the light-harvesting capacity under lower light conditions (Dai et al., 2009). Accordingly, Chl a+b was correlated to Chl a/b ratio negatively and P_n per leaf area positively (Fig. 6).

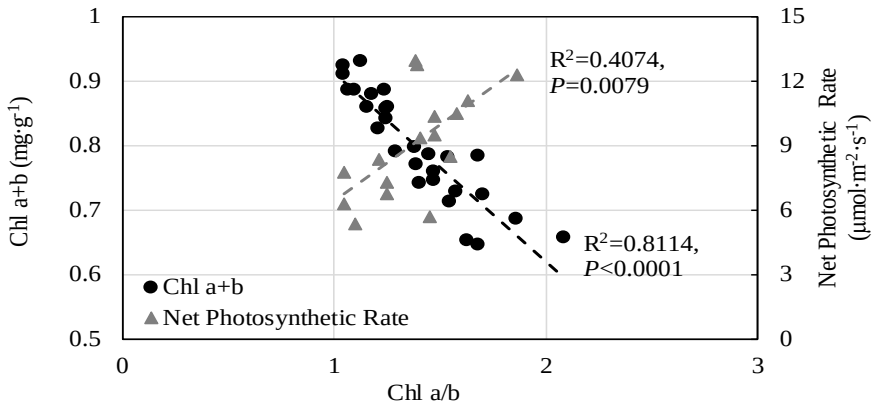

Figure 6. Correlation between Chl a+b with Chl a/b ratio and correlation between net photosynthetic rate per leaf area with Chl a/b ratio of 'Improved Genovese Compact' basil plants grown for 21 days at different daily light integrals (DLIs). Dash lines show regression between measured parameters and Chl a/b ratio according to Pairwise Correlation method. Adapted with permission from Dou et al. (2018).

Plant photosynthetic rate per leaf area depends not only on photosynthetic biochemistry but also on the mesophyll structure of leaves (Retkute et al., 2015). Since resistance to CO_2 diffusion from the sub-stomatal cavity to the stroma is substantial, mesophyll structure affects P_n by affecting the diffusion of CO_2 (Terashima et al., 2001) and the penetration of light in leaves (Vogelmann and Martin, 1993). Increased G_s under higher DLIs indicated that basil leaves were able to open their stomata much wider than plants grown under lower DLIs, which increased E accordingly (Table 2). This certainly appears to be an important factor for increased P_n under higher DLIs (Table 2, Fig. 5).

Basil leaves developed in lower DLIs are thinner and smaller than those growing in higher DLIs (Table 4), which reduced the respiratory cost of basil leaves to help compensate for the greatly decreased photosynthetic capacity (Dai et al., 2009). Meanwhile, mesophyll cells of basil leaves under higher DLIs are more compact (associated with higher dry matter content) than plants grown under lower DLIs (Fig. 3B). Under lower DLIs, decreased P_n produced insufficient ATPs with low carbon fixation and carbohydrate biosynthesis, resulting in smaller plant canopy (Table 3) and decreased shoot and root FW/DW (Fig. 2). Accordingly, the shortage of photo-assimilate supplies and inadequate sucrose synthesis led to a reduction of soluble sugar content (Table 5) compared to plants grown under higher DLIs.

2.5.2 Enhanced nutritional quality of basil plants under higher DLIs

Plant leaves adapt to light conditions not only anatomically and morphologically, but also biochemically. In addition to stimulating photosynthetic pigments, high DLIs also

stimulate the biosynthesis and accumulation of non-photosynthetic pigments and antioxidants, e.g., anthocyanins, phenolics, and flavonoids, (Albert et al., 2009; Bian et al., 2015; Cominelli et al., 2008; Dou et al., 2017; Wu et al., 2007), acting as screens to reduce excess light received by photosynthetic apparatuses (Logan et al., 2015). All these pigments and antioxidants have generated significant interest among consumers and researchers due to their health-promoting properties and considerable antioxidant potential in preventing cardiovascular and chronic diseases (Colonna et al., 2016; Khanam et al., 2012).

Synthesis of phenolic compounds including phenolic acids, flavonoids, and anthocyanins is enhanced under strong UV and visible light conditions (Takahashi and Badger, 2011; Winkel-Shirley, 2002). Across a range of plant species, phenolic compounds act as light attenuators, light-screening, and photoprotective roles, which are supported by a large body of experimental evidences (Agati and Tattini, 2010; Akula and Ravishankar, 2011; Gould et al., 2010; Hatier et al., 2013; Solovchenko, 2010). For example, purple basil 'Red Rubin' had lower metabolic cost of photoprotective mechanisms and higher biomass increase than green basil 'Tigullio' when being moved from 30% to 100% sunlight, which means purple basil with more anthocyanins was more tolerant of higher DLIs than green basil plants (Tattini et al., 2014). Corroborating existing empirical studies and theoretical predictions, total anthocyanin content of basil leaves was positively influenced by DLIs (Fig. 4A). It was also reported that flavonoids play a more important role than xanthophylls in protecting arabidopsis (*Arabidopsis thaliana*) leaves from long-term visible light-induced oxidative damage (Havaux and Kloppstech, 2001).

Total phenolic and flavonoid concentration of basil leaves were both enhanced under higher DLIs (Table 5), and total phenolic and flavonoid content of basil plants were positively correlated with DLIs (Fig. 4B). Similarly, petunia [*Petunia axillaris* x (*Petunia axillaris* x *Petunia hybrida* cv. 'Rose of Heaven')] plants displayed intense anthocyanin content throughout the leaves and stems when grown under 750 $\mu mol \cdot m^{-2} \cdot s^{-1}$ compared to 50-350 $\mu mol \cdot m^{-2} \cdot s^{-1}$, as well as the activation of the early and late flavonoid biosynthetic genes required for flavonoids and anthocyanin production (Albert et al., 2009).

Antioxidant capacity is an important parameter in assessing the quality of fresh herbs, since antioxidant molecules play a fundamental role in inhibiting the formation of free radicals in both plants and humans (Khanam et al., 2012). The enrichment of potent antioxidants, namely, anthocyanins, phenolics, and flavonoids, resulted in higher antioxidant capacity of basil leaves grown under higher DLIs (Table 5).

2.5.3 Future research perspectives

This experiment was conducted at five DLIs created by growing basil plants under five different light intensities with the same 16-h photoperiod. As one factor of the lighting conditions, photoperiod also influences leaf expansion, plant yield, and nutritional content accumulation of plants (Beaman et al., 2009). Few studies on responses of basil plants to different photoperiods in indoor controlled environment were published since it is believed that basil is a long-day plant, and a 16-h photoperiod was used in most studies on basil cultivation in IVFs (Beaman et al., 2009; Piovene et al., 2015). However, what are the responses of basil plants to DLIs created by different photoperiods with the same light

intensity? Furthermore, what are the responses of basil plants to different combinations of light intensity and photoperiod at the same optimal DLI? These might be the future research perspectives.

2.6 Conclusion

Under indoor controlled environment, basil plants grown under higher DLIs had increased photosynthetic capacity per unit leaf area, Chl content, leaf DW, and higher Chl a/b ratios than plants grown under lower DLIs. Higher photosynthetic capacity resulted in larger canopy and branching, larger and thicker leaves, greater leaf and shoot yield, as well as higher dry matter content under DLIs of 12.9, 16.5, and 17.8 $mol \cdot m^{-2} \cdot d^{-1}$, compared to 9.3 and 11.5 $mol \cdot m^{-2} \cdot d^{-1}$. Meanwhile, nutritional contents of basil leaves (soluble sugar, anthocyanin, phenolic compounds, and flavonoids) were positively correlated with DLI, and antioxidant capacity of basil leaves at DLI of 17.8 $mol \cdot m^{-2} \cdot d^{-1}$ was 73% higher than 9.3 $mol \cdot m^{-2} \cdot d^{-1}$. Combining results in growth, yield, and nutritional quality of basil plants, we suggest a DLI of 12.9 $mol \cdot m^{-2} \cdot d^{-1}$ for basil commercial production in indoor vertical farming to minimize the energy cost while maintain a high yield and nutritional quality.

CHAPTER III

PHOTOSYNTHESIS, GROWTH, AND SECONDARY METABOLITES ACCUMULATION IN BASIL, KALE, AND MUSTARD PLANTS UNDER DIFFERENT PROPORTIONS OF RED, BLUE, AND GREEN LIGHT

3.1 Synopsis

Effects of light quality on plant growth and nutritional quality were evaluated on culinary herbs and leafy greens, including basil (*Ocimum basilicum*) 'Improved Genovese Compact' (green) and 'Red Rubin' (purple), green kale 'Siberian' (*Brassica napus pabularia*), red kale 'Scarlet' (*Brassica oleracea*), green mustard 'Amara' (*Brassica carinata*), and red mustard 'Red Giant' (*Brassica juncea*). There were five light quality treatments including three combined red and blue (R&B) light emitting diode (LED) lights with different blue light proportions, $R_{88}B_{12}$ (the percentage of red and blue light was 88% and 12%, respectively), $R_{76}B_{24}$, and $R_{51}B_{49}$, and two red and blue and green (R&B&G) light (additional green light to combined R&B light), $R_{43}B_{13}G_{44}$ and $R_{34}B_{25}G_{41}$ applied to plants with the same photosynthetic photon flux density (PPFD) at 224 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 16-h photoperiod. Plants were sub-irrigated as needed using a nutrient solution with electrical conductivity of 2.0 $dS \cdot m^{-1}$ and pH of 6.0. Results indicated that increase of blue light proportions from 12% to 49% increased net photosynthetic rate and chlorophyll content in purple basil plants by 30% and 10%, respectively, while higher red light proportions increased plant height, leaf area, and subsequently plant yield in all plant species except red mustard plants. Additional green light decreased net photosynthetic rate

and chlorophyll content in red kale and purple basil plants, respectively, compared to combined R&B light treatments with similar blue light proportions. Meanwhile, additional green light increased plant height in green basil and green mustard plants at low blue light proportion of 12%, while decreased plant height in purple basil, green kale, red kale, and green mustard plants at high blue light proportion of 24%. Increases of blue light proportions induced synthesis of secondary metabolites. Effects of additional green light on plant secondary metabolites accumulation is species specific, which decreased concentrations of phenolics and flavonoids in basil plants but increased phenolics concentration in green kale plants.

3.2 Introduction

In recent years, food production under controlled environment, especially crop production in indoor vertical farms (IVFs), has been drawing a lot of attention due to increasing world population and urbanization, global climate change, competition of resources (e.g. land, water, and energy), and increasing demand of local and fresh food with high quality (Despommier, 2013; Kozai et al., 2015; Tornaghi, 2017). Light is a key environmental factor affecting plant growth, development, and secondary metabolism, and artificial lighting is one of the largest operation-cost factors in IVFs (Dou et al., 2017; Dou et al., 2018; Hosseini et al., 2018). Therefore, choosing the optimal light would significantly reduce the production cost in IVFs while improve plant biomass productivity and enhance bioactive secondary metabolites accumulation (Kozai et al., 2015). Recently, LED light has become a widely used light source in IVFs because of its high energy

utilization efficiency, spectra specificity, low surface temperature, long durability, and higher operating capability compared to the conventional fluorescent lamps (FLs), incandescent lamps, or high intensity discharge lamps (Mitchell et al., 2015; Stutte, 2009). With the development of LED technology, a number of studies have been conducted to characterize the effects of light quality on plant growth and nutritional quality to optimize the selection of light sources in IVFs (Darko et al., 2014; Ouzounis et al., 2015).

Red and blue lights are the most efficient light wavelengths in plant biomass accumulation affecting plant photosynthesis and photomorphogenesis. Supplemental blue light to dominant red light was reported to achieve greater plant yield, while plants grown under monochromatic red light had physiological disorders (Bondada and Syvertsen, 2003; Li, 2010; Wollaeger and Runkle, 2014). For instance, monochromatic red light decreased F_v/F_m, stomata density, photosynthetic capacity, and impaired growth in cucumber (*Cucumis sativus*) and tomato (*Solanum lycopersicum*) plants, which was defined as the "red light syndrome", and none of these effects occurred in leaves that were grown under combined R&B light (Hogewoning et al., 2010; Savvides et al., 2011; Trouwborst et al., 2016). Furthermore, spinach (*Spinacia oleracea*) and non-heading Chinese cabbage (*Brassica campestris*, 'Te Ai Qing') had greater leaf area, and shoot fresh weight (FW) and dry weight (DW) under combined R&B LED light compared to monochromatic red or blue LED light (Fan et al., 2013; Ohashi-Kaneko et al., 2007).

Although it is clear that combined R&B LED light is more suitable for plant growth and biomass combination, the optimal red or blue light proportions in combined R&B light is still unknown, and studies indicated it is species specific. For instance, when blue

light proportion was increased from 11% to 28% at a PPFD of 500 μmol·m^{-2}·s^{-1} with a 16-h photoperiod, the dry mass in tomato, cucumber, radish (*Raphanus sativus*), and pepper (*Capsicum annum*) plants decreased, while the blue light proportions did not affect dry mass in soybeans (*Glycine max*), lettuce (*Lactuca sativa*) or wheat (*Triticum aestivum*) (Snowden et al., 2016). He et al. (2015) reported that blue light proportion at 16% treatment achieved the highest photosynthetic capacity and biomass productivity for Chinese broccoli (*Brassica alboglabra*) plants grown under combined R&B LED light (blue light proportions ranging from 0% to 24%). However, the shoot FW of basil plants was the highest under combined R&B light with blue light proportion at 59%, and decreased by 16%, 39%, and 68% compared to light treatments with blue light proportions of 48%, 40%, and 15%, respectively (Piovene et al., 2015). Hypotheses on how light quality affecting plant growth were attributed to the effects of red and blue lights on plant photosynthesis and photomorphogenesis. Red light increases total chlorophyll content in plant leaves to promote the gas exchange rate while blue light improves activities of ribulose-1,5-bisphosphate carboxylase and phosphoenolpyruvate carboxylase (Rubisco) and promotes stomatal opening to improve plant photosynthesis (Bondada and Syvertsen, 2003; Li, 2010). Moreover, red light stimulates plant extension growth via phytochromes while blue light inhibits stem elongation and leaf expansion via cryptochromes and phototropins, which regulate plant morphogenesis antagonistically (Anpo et al., 2018; Bugbee, 2016).

Effects of red and blue lights on secondary plant metabolites accumulation depend on plant species and specific phytochemical compounds. For example, concentration of

rosmarinic acid, the major phenolic acid in basil plants, was twice in plants grown under red and white light compared to those grown under monochromatic blue light, while content of chicoric acid, the second major phenolic acid in basil plants, was higher under blue light than red light (Amaki et al., 2011; Shiga et al., 2009; Shoji et al., 2011). Consistently, expression of polyphenol oxidase (PPO), a key metabolism enzyme in the synthesis of phenolics, increased under supplemental red and blue light in lettuce and salvia (*Salvia miltiorrhiza*) plants (Li et al., 2010). Similarly, l-menthol content in Japanese mint (*Mentha arvensis*) plants enhanced under red light, while polyphenol, total antioxidants, anthocyanin and carotenoid concentration in leaf lettuce, and β-carotene and lutein concentrations in spinach plants increased by blue light (Johkan et al., 2010; Li and Kubota, 2009; Nishioka et al., 2008).

Green light is less studied compared to red and blue lights due to its low absorptivity in the absorption spectra of purified chlorophylls. However, in a living leaf or whole plant canopy, the relative quantum efficiency for broadband green light is 0.87, which is slightly lower than that for red light (0.91) and higher than that for blue light (0.73) (Sager et al., 1988). In addition, while red and blue lights are strongly absorbed by the upper level plant canopy, green light penetrates into deeper plant canopy, which could potentially increase plant yield (Terashima et al., 2009; Wang and Folta, 2013). In fact, at the same PPFD of 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 18-h photoperiod, leaf area and shoot FW and DW in 'Waldmann's Green' lettuce grown under $R_{61}B_{15}G_{24}$ (the proportion of red, blue, and green light at 61%, 15%, and 24%, respectively) treatment increased by 31%, 45%, and 47% compared to plants grown under $R_{84}B_{16}$ treatment, respectively, indicating additional

green light at a similar blue light proportion could increase plant biomass accumulation (Kim et al., 2004). Moreover, additional green light to combined R&B light would make plant appear normal green color instead of purplish, which makes visual assessment of physiological disorders easy, also offer psychological benefit to the farm workers. However, some researchers reported that green light reverses blue or red light induced responses, which played a negative role or have no effects on plant photosynthesis or growth (Folta and Maruhnich, 2007; Talbott et al., 2006). For instance, net photosynthetic rate (P_n) and chlorophyll concentration of 'Green Skirt' lettuce decreased by red, blue and green (R&B&G) light compared to combined R&B LED lights with the same blue light proportion at a PPFD of 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 16-h photoperiod, while the combined R&B&G light showed no effects on the leaf length or width (Kang et al., 2016). As green light proportion increased, anthocyanin concentrations in arabidopsis (*Arabidopsis thaliana*) and 'Red Sails' lettuce plants decreased significantly (Zhang and Folta, 2012; Zhang et al., 2011). It is also reported that stomatal opening stimulated by blue light was reversed by green light in a range of plant species, and additional green light to R&B LEDs induced the shade growth symptoms in arabidopsis plants (Frechilla et al., 2000; Talbott et al., 2002; Zhang and Folta, 2012).

Fore-research indicated that a certain amount of blue light is required for normal plant growth and secondary metabolites accumulation, but responses of individual species to different blue light proportions in combined R&B light is still an ongoing discussion, as well as the effects of green light on plant photosynthesis and photomorphogenesis. Basil

and *Brassica* plants are highly diverse in species and cultivars and are a valuable part of human diet owing to their relatively high levels of bioactive secondary metabolites (Keservani et al., 2010; Makri and Kintzios, 2008; Qian et al., 2016). Therefore, the objective of this study was to investigate the effects of different blue light proportions and additional green light to combined R&B lights on photosynthesis, morphology, yield, and secondary metabolism in culinary herbs (green and purple leaf basil plants) and leafy greens (green and red leaf kale, and mustard plants), overall leading to the definition of optimal light composition for increasing yield and nutritional quality in culinary herbs and leafy greens cultivated in IVFs.

3.3 Materials and Methods

3.3.1 Plant materials and growing conditions

Six plant species including basil (*Ocimum basilicum*) 'Improved Genovese Compact' (green) and 'Red Rubin' (purple), green mustard 'Amara' (*Brassica carinata*), red mustard 'Red Giant' (*Brassica juncea*), green kale 'Siberian' (*Brassica napus pabularia*), and red kale 'Scarlet' (*Brassica oleracea*) (Johnny's Selected Seeds, Winslow, ME, USA) were studied in a walk-in growth room in Texas A&M AgriLife Research and Extension Center at El Paso, TX. For all experiments, one seed per cell was sown in 72 square cell trays (cell size: 3.86 cm L × 5.72 cm H, with a volume of 59 cm^3) with Metro-Mix 360 (peat moss 41%, vermiculite 34%, pine bark 25%, Sun Gro® Horticulture, Bellevue, WA, USA). All trays were placed under mist in a greenhouse for germination. Seedlings were moved from mist after the emergence of cotyledon and grown in a greenhouse until

transplanting. With one pair of true leaves expanded, plant seedlings were transplanted into square pots (pot size: 9.52 cm L × 8.26 cm H, with a volume of 574 cm^3) with Metro-Mix 360, and uniform plants were selected and moved to the walk-in growth room for different light quality treatments described as below.

3.3.2 Light quality treatments

There were five light quality treatments in total, including three combined R&B LED lights with different blue light proportions and two R&B&G light (additional green light to combined R&B light). Three combined R&B LED lights were $R_{88}B_{12}$ (the percentage of red and blue light was 88% and 12%, respectively, Model GEHL48HPPR, Hort Americas, Bedford, TX, USA), $R_{76}B_{24}$ (Model GEHL48HPPB), and $R_{51}B_{49}$ (Model GEHL48HPPV) treatments. Two R&B&G light treatments were $R_{43}B_{13}G_{44}$ (white LED light, in which the red, blue, and green light percentage is 43%, 13%, and 44%, respectively, Model GEHL48HWTB), and $R_{34}B_{25}G_{41}$ (white fluorescent light, Philips Lighting, Somerset, NJ, USA) treatments. All treatments were maintained at the same PPFD level at 224 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 16-h photoperiod. The light spectrum distribution in all experiments was measured at 15 cm underneath the light at 9 spots using PS-100 spectroradiometer (Apogee Instruments, Logan, UT, USA) before placing the plants (Fig. 7). To minimize light distribution being disproportionate within each treatment, all plants were systematically rearranged every three days.

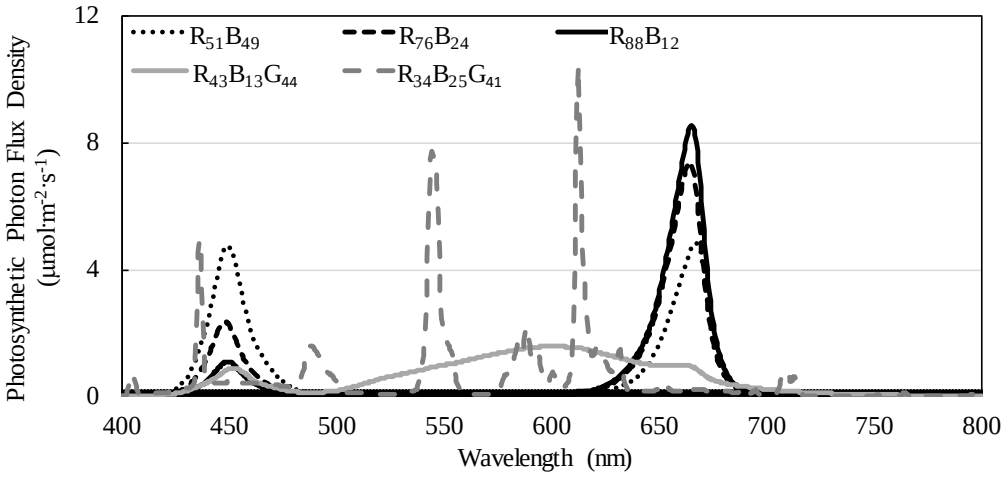

Figure 7. Light spectrum distribution of different light quality treatments including $R_{88}B_{12}$ (combined red (R) and blue (B) LED light, in which the red and blue light percentage is 88% and 12%, respectively), $R_{76}B_{24}$, $R_{51}B_{49}$, $R_{43}B_{13}G_{44}$, and $R_{34}B_{25}G_{41}$ treatments. Photosynthetic photon flux density and light spectrum distribution was measured using a PS-100 spectroradiometer.

After transplanting, all plants were sub-irrigated as needed with nutrient solution containing 1.85 $g \cdot L^{-1}$ (277.5 ppm N) 15N-2.2P-12.5K (Peters 15-5-15 Ca-Mg Special, The Scotts Company, Marysville, OH, USA), maintaining electrical conductivity of 2.0 $dS \cdot m^{-1}$ and pH of 6.0. Mechanical mini fans (LS1225A-X, AC Infinity, City of Industry, CA, USA) were used to circulate the air to achieve uniform temperatures across treatments. Plant canopy temperatures in each experiment were recorded by a data logger (CR1000, Campbell Scientific, Logan, UT, USA) and maintained at 23.5/21.3ºC, 23.4/20.2ºC, and 22.5/20.0ºC day/night for basil, mustard, and kale plants, respectively.

All plants were harvested when plant height reached about 25 cm. The green and purple basil plants were harvested at 21 and 28 days after treatment (DAT), respectively, which meant at 42 and 49 days after sowing (DAS), respectively. The green and red kale plants

were harvested at 18 and 25 DAT (32 and 39 DAS), respectively. The green and red mustard were both harvested at 21 DAT (35 DAS). In each experiment, there were 18, 10, and 10 basil, kale, and mustard plants per treatment per cultivar, respectively.

3.3.3 Measurements

1. Gas-exchange and chlorophyll content

A portable gas-exchange analyzer (CIRAS-3, Portable Photosynthesis Systems International, Amesbury, MA, USA) was used to measure P_n of plant leaves at harvest. A PLC3 leaf cuvette with LED light unit was used, and PPFD, relative air humidity, and CO_2 concentration inside the leaf chamber were kept constant at 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 50%, and 390 $\mu mol \cdot mol^{-1}$, respectively. Five plants per treatment per cultivar were randomly selected for measurement.

The soil plant analysis development (SPAD) index of plant leaves was recorded at harvest to quantify the relative chlorophyll content in plant leaves using a chlorophyll meter SPAD-502 (Konica-Minolta cooperation, Ltd., Osaka, Japan).

2. Growth parameters

Growth characteristics including plant height and leaf area were recorded at the end of the experiment. Crop yield including shoot FW and DW were also measured at the end of the experiment. Five plants per treatment were randomly selected for measurement. Leaf area was measured using a leaf area meter (LI-3100, LI-COR, Lincoln, NE, USA), and the shoot tissues were dried at 80ºC in a drying oven (Grieve, Round Lake, IL, USA) for 3 days to determine the shoot DW.

3. Secondary plant metabolites measurement

Four plants per treatment per cultivar were randomly selected for measurements of anthocyanin concentration, total phenolic concentration, total flavonoid concentration, and antioxidant capacity of plant leaves at harvest. Fresh leaves were collected in a cooler and immediately stored in a deep freezer (IU1786A, Thermo Fisher Scientific, Marietta, OH, USA) at -80ºC until phytochemical analyses.

Extraction. Approximately 2 g fresh basil leaves were ground in liquid nitrogen and extracted with 15 mL 1% acidified methanol in darkness. After overnight extraction, the mixture was centrifuged (Sorvall RC 6 Plus Centrifuge, Thermo Fisher Scientific, Madison, WI, USA) at 13,200 rpm (26,669 ×*g*) for 15 min, and the supernatant was collected for phytochemical analyses (Xu and Mou, 2016), 2016).

Anthocyanin analysis. The absorbance of extracts was measured at 530 nm using a spectrophotometer (Genesys 10S ultraviolet/ Vis, Thermo Fisher Scientific, Madison, WI, USA), and the anthocyanin concentration was expressed as mg cyanidin-3-glucoside equivalents using a molar extinction coefficient of 29,600 (Connor et al., 2002). Since the extracts were freshly prepared from leaf tissues maintained at -80ºC and did not undergo extensive processing or significant browning, a pH differential method for anthocyanin content was considered unnecessary (Connor et al., 2002).

Phenolics analysis. The total phenolics concentration of basil leaves was determined using the modified Folin-Ciocalteu reagent method described as the following: 100 μL extraction sample was added to a mixture of 150 μL distilled water and 750 μL 1/10 dilution Folin-Ciocalteu reagent. After 6 min reaction, 600 μL 7.5% Na_2CO_3 was added

and the mixture was incubated at 45°C in water bath for 10 min before the absorbance was measured at 725 nm using a microplate reader (ELx800, BioTek, Winooski, VT, USA). Results were expressed as mg of gallic acid equivalent per g FW of basil leaves (Xu and Mou, 2016).

Flavonoids analysis. The total flavonoid concentration of basil leaves was determined as the following: 20 µL extraction sample was added to a mixture of 85 µL distilled water and 5 µL 5% $NaNO_2$. After 6 min reaction, 10 µL of 10% $AlCl_3 \cdot 6H_2O$ was added to the mixture. Five min later, 35 µL of 1M NaOH and 20 µL distilled water were added to the mixture and the absorbance was measured at 520nm using the aforementioned microplate reader (Dou et al., 2018). The results were expressed as mg of (+)-catechin hydrate equivalent per g FW of basil leaves.

Antioxidant capacity analysis. The antioxidant capacity of basil leaves was measured using the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) method (Arnao et al., 2001) described as the following: add a mixture of 150 µL basil leave extracts to 2.85 mL of $ABTS^+$ solution and incubate at room temperature for 10 min. The absorbance of mixed solution was measured at 734 nm using the aforementioned spectrophotometer. Antioxidant capacity of basil leaves was expressed as mg of Trolox equivalent antioxidant capacity per 100 g FW of basil leaves.

3.3.4 Statistical analysis

One-way analysis of variance was conducted to analyze the effects of light quality on all measured parameters. Multiple means comparison among treatments was conducted

using Student's *t* method. Correlation test was conducted using Pairwise Correlations method. All statistical analyses were performed using JMP software (Version 13, SAS Institute Inc., Cary, NC, USA), and a $P < 0.05$ was considered as significant.

3.4 Results

3.4.1 Gas exchange rate and chlorophyll content as influenced by red, blue, and green light

1. Green and purple basils

Net photosynthetic rate and SPAD readings of green basil plants showed no differences amongreen light quality treatments (Tables 6, 7), while in purple basil plants, both parameters were the highest under $R_{51}B_{49}$ treatment, which increased by 15%-34% and 10%-24% compared to other treatments, respectively. In purple basil plants, P_n was positively correlated with blue light proportions with a coefficient of 0.9107. Additional green light showed no effects on P_n in purple basil plants compared to plants grown under combined R&B light with similar blue light proportion, while it decreased SPAD readings (Tables 6, 7).

Table 6. Net photosynthetic rate of green basil 'Improved Genovese Compact', purple basil 'Red Rubin', green kale 'Siberian', red kale 'Scarlet', green mustard 'Amara', and red mustard 'Red Giant' plants under different light quality treatments.

Treatment	BP (%)	Net Photosynthetic Rate ($\mu mol \cdot m^{-2} \cdot s^{-1}$)					
		Green Basil	Purple Basil	Green Kale	Red Kale	Green Mustard	Red Mustard
$R_{88}B_{12}$	12	10.7 a[z]	6.3 b	11.0 b	12.5 ab	19.2 a	17.8 a
$R_{43}B_{13}G_{44}$	13	11.1 a	6.1 b	10.5 b	11.5 bc	15.2 a	17.9 a
$R_{76}B_{24}$	24	10.1 a	6.1 b	12.3 ab	13.4 a	18.1 a	18.0 a
$R_{34}B_{25}G_{41}$	25	11.5 a	7.1 ab	11.8 b	10.1 c	16.0 a	15.2 a
$R_{51}B_{49}$	49	12.9 a	8.2 a	13.2 a	12.5 ab	19.3 a	16.9 a
ANOVA		NS	*	*	**	NS	NS
Correlation Test	γ	-	0.9107	0.9279	-	-	-
	P	NS	*	*	NS	NS	NS

[z] Means followed by different lowercase letters indicate significant difference, according to Student's t mean comparison ($P < 0.05$).

Asterisks (*) indicate significant differences (*$P < 0.05$; **$P < 0.01$). NS indicates means are not significantly, or correlation between net photosynthetic rate and blue light proportions (BPs) is not significant ($P < 0.05$).

Correlation test between net photosynthetic rate and BPs was conducted using Pairwise Correlation method, and coefficient (γ) is presented when correlation is significant.

Table 7. Soil plant analysis development (SPAD) of green basil 'Improved Genovese Compact', purple basil 'Red Rubin', green kale 'Siberian', red kale 'Scarlet', green mustard 'Amara', and red mustard 'Red Giant' plants under different light quality treatments.

Treatment	BP (%)	SPAD					
		Green Basil	Purple Basil	Green Kale	Red Kale	Green Mustard	Red Mustard
$R_{88}B_{12}$	12	37.9 a[z]	41.9 b	44.3 a	46.2 c	48.0 a	37.2 a
$R_{43}B_{13}G_{44}$	13	40.2 a	37.2 c	43.9 a	53.1 a	50.0 a	36.9 a
$R_{76}B_{24}$	24	40.2 a	45.6 a	44.0 a	50.6 ab	49.7 a	34.7 a
$R_{34}B_{25}G_{41}$	25	36.5 a	41.4 b	47.6 a	48.1 bc	49.0 a	37.8 a
$R_{51}B_{49}$	49	37.7 a	46.1 a	45.2 a	53.2 a	50.1 a	36.2 a
ANOVA		NS	*	NS	*	NS	NS
Correlation Test	γ	-	-	-	-	-	-
	P	NS	NS	NS	NS	NS	NS

[z] Means followed by different lowercase letters indicate significant difference, according to Student's t mean comparison ($P < 0.05$).
Asterisks (*) indicate significant differences ($*P < 0.05$). NS indicates means are not significantly different among treatments, or correlation between tested parameters and blue light proportions (BPs) is not significant ($P < 0.05$).
Correlation tests between tested parameters and BPs were conducted using Pairwise Correlation method, and coefficient (γ) is presented when correlation is significant.

2. Green and red kales

Net photosynthetic rate of green kale plants was the highest under $R_{51}B_{49}$ treatment and was positively correlated with blue light proportions with a coefficient of 0.9279 (Table 6). In red kale plants, P_n was not influenced by blue light proportions among three combined R&B LED treatments, while additional green light significantly decreased P_n regardless of blue light proportions (Table 6). SPAD readings of green kale plants were not affected by light quality treatments. Increases of blue light proportions increased the SPAD readings in red kale plants among combined R&B LED treatments. At a blue light

proportion at 12%, additional green light increased the SPAD readings in red kale plants by 15%, while additional green light showed no effects at a blue light proportion at 24% (Table 7).

3. Green and red mustards

No differences were observed on P_n or SPAD readings in green or red mustard plants among different light quality treatments (Tables 6, 7).

3.4.2 Growth parameters and crop yield as influenced by red, blue, and green light

1. Green and purple basils

Without additional green light, plant height in green and purple basil plants was the lowest under $R_{51}B_{49}$ treatment. Additional green light increased plant height of green basil plants at a blue light proportion at 12%, while it showed no effects in purple basil plants. In contrary, at a blue light proportion at 24%, additional green light showed no effects on plant height in green basil plants but decreased it in purple basil plants (Table 8). Leaf area in green and purple basil plants both decreased with increasing blue light proportions among combined R&B LED treatments, while additional green light decreased leaf area by 8%-44% regardless of cultivar (Table 8). No correlations between growth parameters (i.e. plant height and leaf area) and blue light proportions were observed in green or purple basil plants (Table 9). Similarly, shoot FW and DW in green and purple basil plants both decreased with increasing blue light proportions among the three combined R&B LED treatments. Additional green light decreased the shoot FW and DW by 27%-41% in purple basil plants compared to combined R&B LED lights with similar blue light proportions,

while it decreased the shoot FW in green basil plants at a blue light proportion at 12% (Table 9). No correlations between plant yield (i.e. shoot FW and DW) and blue light proportions were observed in green or purple basil plants (Table 9).

Table 8. Plant height and leaf area of green basil 'Improved Genovese Compact', purple basil 'Red Rubin', green kale 'Siberian', red kale 'Scarlet', green mustard 'Amara', and red mustard 'Red Giant' plants under different light quality treatments.

Treatment	BP (%)	Plant Height (cm)					
		Green Basil	Purple Basil	Green Kale	Red Kale	Green Mustard	Red Mustard
$R_{88}B_{12}$	12	24.3 b[z]	18.3 ab	33.8 a	25.2 a	24.8 b	28.1 a
$R_{43}B_{13}G_{44}$	13	26.1 a	17.4 ab	34.9 a	25.2 a	26.6 a	27.6 a
$R_{76}B_{24}$	24	22.5 c	18.9 a	32.2 ab	25.2 a	24.2 b	28.1 a
$R_{34}B_{25}G_{41}$	25	22.1 c	17.3 b	27.9 c	22.0 b	22.7 c	26.3 a
$R_{51}B_{49}$	49	22.1 c	17.2 b	30.5 bc	20.8 b	21.1 d	28.3 a
ANOVA		***	*	**	***	***	NS
Correlation Test	γ	-	-	-	-	-0.8958	-
	P	NS	NS	NS	NS	*	NS
		Leaf Area (cm^2)					
$R_{88}B_{12}$	12	669 a	631 a	817 a	660 a	697 a	812 a
$R_{43}B_{13}G_{44}$	13	605 ab	356 d	820 ab	631 ab	658 ab	735 ab
$R_{76}B_{24}$	24	606 ab	552 b	708 bc	615 ab	626 abc	829 a
$R_{34}B_{25}G_{41}$	25	560 b	407 cd	637 c	536 bc	574 bc	576 b
$R_{51}B_{49}$	49	546 b	456 c	625 c	505 c	547 c	782 a
ANOVA		*	***	**	*	*	*
Correlation Test	γ	-	-	-	-0.8831	-0.8844	-
	P	NS	NS	NS	*	*	NS

[z] Means followed by different lowercase letters indicate significant difference, according to Student's t mean comparison ($P < 0.05$).

Asterisks (*) indicate significant differences (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). NS indicates means are not significantly different among treatments, or correlation between tested parameters and blue light proportions (BPs) is not significant ($P < 0.05$).

Correlation tests between tested parameters and BPs were conducted using Pairwise Correlation method, and coefficient (γ) is presented when correlation is significant.

Table 9. Shoot fresh weight of green basil 'Improved Genovese Compact', purple basil 'Red Rubin', green kale 'Siberian', red kale 'Scarlet', green mustard 'Amara', and red mustard 'Red Giant' plants under different light quality treatments.

Treatment	BP (%)	Shoot Fresh Weight (g)					
		Green Basil	Purple Basil	Green Kale	Red Kale	Green Mustard	Red Mustard
$R_{88}B_{12}$	12	25.0 a[z]	19.4 a	49.4 ab	35.8 a	45.6 a	48.9 a
$R_{43}B_{13}G_{44}$	13	21.3 b	12.7 c	51.1 a	32.2 ab	43.3 ab	43.5 a
$R_{76}B_{24}$	24	21.7 b	18.8 a	47.8 ab	33.9 a	39.3 bc	49.5 a
$R_{34}B_{25}G_{41}$	25	20.2 b	13.6 bc	37.9 c	24.3 c	35.6 c	33.5 b
$R_{51}B_{49}$	49	20.1 b	15.5 b	40.5 bc	27.2 bc	35.5 c	46.4 a
ANOVA		*	***	*	**	**	*
Correlation Test	γ	-	-	-	-	-	-
	P	NS	NS	NS	NS	NS	NS
		Shoot Dry Weight (g)					
$R_{88}B_{12}$	12	1.91 a	1.53 a	3.5 a	3.8 ab	2.94 a	2.79 a
$R_{43}B_{13}G_{44}$	13	1.75 ab	0.91 d	3.5 a	4.4 a	2.80 a	2.43 ab
$R_{76}B_{24}$	24	1.63 b	1.47 a	3.1 a	3.7 abc	2.56 a	2.90 a
$R_{34}B_{25}G_{41}$	25	1.51 b	1.08 c	2.7 a	2.7 c	2.59 a	2.06 b
$R_{51}B_{49}$	49	1.56 b	1.28 b	2.7 a	2.9 bc	2.64 a	2.81 a
ANOVA		**	***	NS	*	NS	*
Correlation Test	γ	-	-	-	-	-	-
	P	NS	NS	NS	NS	NS	NS

[z] Means followed by different lowercase letters indicate significant difference, according to Student's t mean comparison ($P < 0.05$).
Asterisks (*) indicate significant differences (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). NS indicates means are not significantly different among treatments, or correlation between tested parameters and blue light proportions (BPs) is not significant ($P < 0.05$).
Correlation tests between tested parameters and BPs were conducted using Pairwise Correlation method.

2. Green and red kales

Plant height and leaf area of green and red kale plants decreased with increasing blue light proportions among the combined R&B LED treatments and additional green light decreased plant height and leaf area at a blue light proportion at 24% regardless of cultivar (Table 8). Leaf area in red kale plants was negatively correlated with blue light proportions

with a coefficient of -0.8831 (Table 8). Shoot FW of green and red kale plants had a similar trend as plant height and leaf area (Table 9). Shoot FW of green and red kale plants increased by 22% and 32% with decreasing blue light proportions from 49% to 12%. Shoot DW of red kale plants had a similar trend as the shoot FW, while different light quality had no influence on the shoot DW of green kale plants. No correlations between plant yield and blue light proportions were observed in green or red basil plants (Table 9).

3. Green and red mustards

Plant height and leaf area of green mustard plants were both negatively correlated with blue light proportions with a coefficient of -0.8958 and -0.8844, respectively (Table 8). Plant height and leaf area in green mustard plants increased by 17% and 27% with decreasing blue light proportions from 49% to 12%, respectively. Additional green light increased plant height of green mustard plants at a blue light proportion at 12% but decreased it at a blue light proportion at 24%. Light quality treatments showed no influence on plant height in red mustard plants, while additional green light decreased its leaf area at a blue light proportion at 24% (Table 8). No correlations between growth parameters and blue light proportions were observed in red mustard plants (Table 8). Shoot FW of green mustard plants decreased with increasing blue light proportions, while the shoot FW of red mustard plants only decreased with additional green light at a blue light proportion at 24% (Table 9). Similarly, shoot DW of red mustard plants decreased with additional Green light at a blue light proportion at 24%, while different light quality had no influence on the shoot DW in green kale plants. No correlations between crop yield and blue light proportions were observed in mustard plants (Table 9).

3.4.3 Secondary metabolites accumulation as influenced by red, blue, and green light

1. Green and purple basils

Different light quality treatments had no influence on anthocyanin concentration of green or purple basil plants (Table 10). Phenolic concentration in green basil plants was not affected by blue light proportions among combined R&B LED treatments, while additional green light decreased it regardless of blue light proportion (Table 10). In purple basil plants, increases of blue light proportions also increased phenolic concentration, while additional green light decreased it at a blue light proportion at 24%. Flavonoid concentration of green basil plants was the highest under a blue light proportion at 24% and decreased by blue light proportions at 12% and 49%, while in purple basil plants, flavonoid concentration was the lowest under a blue light proportion at 12% and showed no differences between blue light proportions of 24% and 49%. Additional green light decreased flavonoid concentration in purple basil plants regardless of blue light proportion (Table 10). Similarly, antioxidant capacity of green and purple basil plants decreased by 11%-30% with additional green light. No correlations between phytochemical parameters and blue light proportions were observed in green or purple basil plants (data not shown).

Total amount of anthocyanin, phenolics, flavonoids, and antioxidant capacity per plant was calculated by multiplying the concentrations of each parameter with leaf FW per plant. Among combined R&B LED treatments, the total amount of anthocyanin, phenolics, flavonoids, and antioxidant capacity in both basil cultivars were all the highest under a blue light proportition at 24%, while decreased by a higher blue light proportion

at 49% (Table 11). Additional green light decreased the total amount of phytochemicals per plant regardless of cultivar or blue light intensity except the total amount of anthocyanin in green basil plants (Table 11).

Table 10. Concentrations of anthocyanin, phenolics, and flavonoids and antioxidant capacity in green basil 'Improved Genovese Compact', purple basil 'Red Rubin', green kale 'Siberian', red kale 'Scarlet', green mustard 'Amara', and red mustard 'Red Giant' plants grown under different light quality treatments with different blue light proportions (BPs).

Treatment	BP (%)	Green Basil	Purple Basil	Green Kale	Red Kale	Green Mustard	Red Mustard
		Anthocyanin Concentration ($mg \cdot 100g^{-1}$)					
$R_{88}B_{12}$	12	2.54 a[z]	13.5 a	6.74 b	9.73 a	7.22 a	9.93 ab
$R_{43}B_{13}G_{44}$	13	2.77 a	14.1 a	7.51 a	9.51 a	7.90 a	9.57 b
$R_{76}B_{24}$	24	2.77 a	13.8 a	7.15 ab	9.61 a	7.53 a	9.47 b
$R_{34}B_{25}G_{41}$	25	2.95 a	13.9 a	7.61 a	10.16 a	7.86 a	9.90 ab
$R_{51}B_{49}$	49	2.75 a	14.1 a	7.44 a	9.74 a	7.70 a	10.27 a
		Phenolics Concentration ($mg \cdot g^{-1}$)					
$R_{88}B_{12}$	12	1.28 a	2.81 c	0.75 c	1.40 bc	0.98 c	1.22 a
$R_{43}B_{13}G_{44}$	13	1.04 b	2.67 c	0.79 bc	1.22 c	1.12 b	1.09 a
$R_{76}B_{24}$	24	1.37 a	3.21 b	0.81 bc	1.47 ab	1.13 b	1.21 a
$R_{34}B_{25}G_{41}$	25	1.01 b	2.62 c	1.11 a	1.65 a	1.12 b	1.27 a
$R_{51}B_{49}$	49	1.30 a	3.47 a	0.98 ab	1.64 a	1.25 a	1.26 a
		Flavonoids Concentration ($mg \cdot g^{-1}$)					
$R_{88}B_{12}$	12	0.49 bc	2.43 b	0.97 a	1.22 a	0.87 a	0.86 a
$R_{43}B_{13}G_{44}$	13	0.48 bc	1.92 c	1.01 a	1.17 a	0.96 a	0.85 a
$R_{76}B_{24}$	24	0.84 a	2.70 a	0.97 a	1.26 a	0.96 a	0.87 a
$R_{34}B_{25}G_{41}$	25	0.40 c	1.87 c	1.07 a	1.13 a	0.95 a	0.85 a
$R_{51}B_{49}$	49	0.53 b	2.70 a	1.04 a	1.22 a	0.90 a	0.90 a
		Antioxidant Capacity ($mg \cdot 100g^{-1}$)					
$R_{88}B_{12}$	12	295 b	1570 b	141 bc	279 a	176 a	221 ab
$R_{43}B_{13}G_{44}$	13	290 bc	1394 c	112 c	210 b	151 a	146 b
$R_{76}B_{24}$	24	389 a	1755 a	121 bc	294 a	186 a	213 ab
$R_{34}B_{25}G_{41}$	25	249 c	1229 d	178 a	314 a	172 a	232 a
$R_{51}B_{49}$	49	251 bc	1873 a	151 ab	334 a	198 a	220 a

[z] Means followed by the same lowercase letters are not significantly different, according to Student's *t* mean comparison ($P < 0.05$).

Table 11. Total amount of anthocyanin, phenolics, and flavonoids and antioxidant capacity per plant in green basil 'Improved Genovese Compact', purple basil 'Red Rubin', green kale 'Siberian', red kale 'Scarlet', green mustard 'Amara', and red mustard 'Red Giant' plants grown under different light quality treatments with different blue light proportions (BPs).

Treatment	BP (%)	Green Basil	Purple Basil	Green Kale	Red Kale	Green Mustard	Red Mustard
		Total Amount of Anthocyanin (mg·plant^{-1})					
$R_{88}B_{12}$	12	0.51 a[z]	2.01 a	3.33 b	3.48 a	3.14 b	4.70 a
$R_{43}B_{13}G_{44}$	13	0.46 ab	1.39 c	3.84 a	3.06 c	3.42 a	4.16 b
$R_{76}B_{24}$	24	0.49 ab	2.01 a	3.42 b	3.26 b	2.96 bc	4.85 a
$R_{34}B_{25}G_{41}$	25	0.48 ab	1.46 c	2.89 c	2.47 e	2.80 cd	3.32 c
$R_{51}B_{49}$	49	0.44 b	1.71 b	3.01 c	2.65 d	2.67 d	4.76 a
		Total Amount of Phenolics (mg·plant^{-1})					
$R_{88}B_{12}$	12	25 a	42 b	37 a	50 a	43 b	58 a
$R_{43}B_{13}G_{44}$	13	18 c	26 c	40 a	39 b	49 a	47 b
$R_{76}B_{24}$	24	24 ab	47 a	39 a	50 a	45 ab	62 a
$R_{34}B_{25}G_{41}$	25	17 c	27 c	42 a	40 b	40 b	43 b
$R_{51}B_{49}$	49	21 b	42 b	40 a	45 ab	42 b	59 a
		Total Amount of Flavonoids (mg·plant^{-1})					
$R_{88}B_{12}$	12	10 b	36 b	48 ab	44 a	38 ab	41 ab
$R_{43}B_{13}G_{44}$	13	8 bc	19 d	52 a	38 b	41 a	37 b
$R_{76}B_{24}$	24	15 a	39 a	46 abc	43 a	38 ab	42 a
$R_{34}B_{25}G_{41}$	25	7 c	20 d	41 c	27 d	34 bc	29 c
$R_{51}B_{49}$	49	9 b	33 c	42 bc	33 c	31 c	42 a
		Total Amount of Antioxidant Capacity (mg·plant^{-1})					
$R_{88}B_{12}$	12	59 b	234 b	70 a	100 a	76 a	103 a
$R_{43}B_{13}G_{44}$	13	49 c	138 c	57 a	68 c	65 a	63 b
$R_{76}B_{24}$	24	68 a	255 a	58 a	100 a	74 a	101 a
$R_{34}B_{25}G_{41}$	25	41 d	129 c	67 a	76 bc	61 a	78 b
$R_{51}B_{49}$	49	40 d	227 b	61 a	91 ab	69 a	102 a

[z] Means followed by the same lowercase letters are not significantly different, according to Student's *t* mean comparison ($P < 0.05$).

2. Green and red kales

Anthocyanin concentration of green kale plants decreased by 9%-11% under $R_{88}B_{12}$ treatment compared to other treatments, while light quality treatments showed no effects

on the anthocyanin concentration in red kale plants (Table 10). Phenolics concentration in green and red kale plants both increased with increasing blue light proportions among combined R&B LED treatments, while additional green light increased phenolics concentration in green kale plants at a blue light proportion at 24%. Flavonoid concentration in green or red kale plants was not affected by light quality treatments. Antioxidant capacity of green kale plants increased with the increases of blue light proportions, while additional green light increased it at a blue light proportion at 24%. Antioxidant capacity in red kale plants was not affected by blue light proportions and decreased with additional green light at a blue light proportion at 12%. No correlations between phytochemical parameters and blue light proportions were observed in green or red kale plants (data not shown).

Among combined R&B LED treatments, changes of blue light proportitions showed no effects on the total amount of phenolics, flavonoids, and antioxidant capacity in green kale plants, or the total amount of phenolics and antioxidant capacity in red kale plants (Table 11). The highest blue light proportion at 49% decreased the total amount of anthocyanin by 10%-24% in both cultivars and decreased the total amount of flavonoids by 25% in red kale plants compared to lower blue light proportion at 12% (Table 11). In green kale plants, addtitional green light treatment increased the total amount of anthocyanin at a lower blue light proportion at 12%, while decreased it at a higher blue light proportion at 24%. Additional green light treatments showed no effects on the total amount of phenolics, flavonoids, or antioxidant capacity per plant in green kale plants. In

red kale plants, additional green light treatment decreased the total amount of phytochemicals per plant despite blue light proportions (Table 11).

3. Green and red mustards

Different light quality treatments had no influence on anthocyanin concentration, flavonoid concentration, or antioxidant capacity in green mustard plants, while its phenolic concentration was positively correlated with blue light proportions with a coefficient of 0.8642 (data not shown). Additional green light increased the phenolic concentration by 14% in green mustard plants at a blue light proportion at 12%. In red mustard plants, light quality treatments showed no effects on concentrations of phenolics or flavonoids (Table 10). Anthocyanin concentration in red mustard plants was the lowest under $R_{76}B_{24}$ treatment and was not influenced by additional green light. Antioxidant capacity of red kale plants was not affected by blue light proportions or additional green light (Table 10).

Among combined R&B LED treatments, the highest blue light proportition at 49% decreased the total amount of anthocyanin and flavonoids in green mustard plants, while changes of blue light proportions showed no effects on the total amount of phytochemicals per plant in red mustard plants (Table 11). In green mustard plants, additional green light treatment increased the total amount of anthocyanin and phenolics at a lower blue light proportion at 12%, while additional green light treatment showed no effects on total amount of phytochemicals at a higher blue light proportion at 24% (Table 11). In red mustard plants, additional green light treatment decreased the total amount of phytochemicals despite blue light proportions (Table 11).

3.5 Discussion

3.5.1 Photosynthesis and chlorophyll content as influenced by red, blue, and green light

Photosynthesis is the basis of plant growth and biomass accumulation, and P_n generally increased with increasing blue light proportions in combined R&B lights (Bugbee, 2016). Consistently in this study, increase of blue light proportions from 12% to 49% significantly increased P_n in purple basil and green kale plants by 30% and 20%, respectively (Table 6). Studies suggested increased P_n under higher blue light proportions could be attributed to blue light enriched chloroplast density, increased stomatal opening, and improved enzyme activity (Fan et al., 2013; Li, 2010). For example, leaf mass per area, chlorophyll concentration, and stomatal conductance in cucumber plants increased at higher blue light proportions (Hernández et al., 2016; Hogewoning et al., 2010). Biosynthetic intermediates of chlorophylls, such as 5-aminolevulinic acid, protoporphyrin IX, Mg-protoporphyrin IX and protochlorophyllide in Chinese cabbage were the lowest under monochromatic red light and increased by blue light addition (Fan et al., 2013). Activities of photosynthetic enzymes, such as Rubisco, and stomatal opening was improved by blue light (Bondada and Syvertsen, 2003; Li, 2010), while monochromatic red light decreased stomatal density and stomatal conductance in cucumber leaves, largely due to a positive effect on epidermal cell size (Hogewoning et al., 2010; Savvides et al., 2011). Consistently, SPAD readings in purple basil and red kale plants increased with increasing blue light proportions from 12% to 49% in the present study (Table 7). It was postulated that increasing blue light proportions could stimulate “high irradiance leaf

characteristics" even under constant irradiance, which improved plant photosynthesis (Hogewoning et al., 2010). Another hypothesis of blue light increased P_n was that red light inhibits the transportation of photosynthate from leaves to sinks, which suppressed the photosynthesis with a high level of carbohydrates in leaves (Bondada and Syvertsen, 2003). However, increases of blue light proportions in other plant species showed no effects on plant photosynthesis or chlorophyll concentration, i.e., changes of blue light proportions from 12% to 49% did not affect P_n in green basil, red kale, green mustard, or red mustard plants, and showed no influence on SPAD readings in green basil, green kale, green mustard, or red mustard plants (Tables 6, 7). He et al. (2015) also reported that increasing blue light proportions from 0% to 24% did not affect chlorophyll and carotenoid concentrations in Chinese broccoli plants. These results suggest that increasing blue light proportions in combined R&B light could improve or have no effects on plant photosynthesis, depending on plant sensitivity.

Additional green light to combined R&B light with similar blue light proportions decreased P_n in red kale plants, and a similar trend was observed in green mustard plants, but not in the other tested plant species (Table 6). This was consistent with previous research results, additional green light repressed or have no effects on plant photosynthesis (Folta and Maruhnich, 2007). Kang et al. (2016) reported that additional green light decreased P_n and chlorophyll concentration in 'Green Skirt' lettuce plants compared to combined R&B light, while P_n in 'Waldmann's Green' lettuce grown under $R_{61}B_{15}G_{24}$ treatment was slightly lower compared to plants grown $R_{84}B_{16}$ treatment, but without significant difference (Kim et al., 2004). Similarly, SPAD readings in purple basil plants

decreased by additional green light when the other plant species were not affected (Table 7). This may be caused by the different sensitivity of plant species to green light (i.e., red kale, green mustard, and purple basil plants) where additional green light reversed the blue light induced stomatal opening and chlorophyll formation, resulting in decreased P_n and SPAD readings, while blue light dominated the photosynthetic responses in other plant species and was not affected by additional green light.

3.5.2 Plant growth and yield as influenced by red, blue, and green light

In the present study, P_n in plants increased with blue light proportions while plant growth rate (i.e., plant height and leaf area) and plant yield decreased with the increases of blue light proportions, indicating that plant photomorphogenesis dominated the biomass accumulation in tested plant species instead of photosynthesis, at least under the experimental setups. Since measured P_n is the instantaneous gas exchange rate on a unit leaf area basis, the total CO_2 assimilation per plant depends largely on plant leaf area, which is positively related with light interception (Kim et al., 2004). Plant expansion growth (stem elongation and leaf expansion) in response to blue light is mediated by dynamic, direct interactions between cryptochromes and phytochromes-interacting factors (PIFs) (Pedmale et al., 2016). With relatively higher blue light proportions in light source, the suppression of PIFs 4 and 5 by cryptochromes and proteasomal degradation of CRY 2 and PIF 5 together inhibit stem elongation and leaf expansion, resulting in reduced light interception (Pedmale et al., 2016). Indeed, plant height of Chinese cabbage was the highest under monochromatic red light, followed by $R_{86}B_{14}$ treatment, and the lowest

under monochromatic blue light at a PPFD of 150 μmol·m^{-2}·s^{-1} with a 12-h photoperiod (Fan et al., 2013). This was consistent with the study presented by Hernández et al. (2016), where plant height, leaf area, and shoot FW and DW in 'Cumlaude' cucumber plants decreased with increasing blue light proportion from 10% to 75% at a PPFD of 100 μmol·m^{-2}·s^{-1}, whereas P_n, stomatal conductance, and chlorophyll concentration increased. Similarly, leaf length and width of 'Green Skirt' lettuce plants decreased with increasing blue light proportion from 0% to 30% at a PPFD of 150 μmol·m^{-2}·s^{-1} with a 16-h photoperiod (Kang et al., 2016). However, contradictory results in plant growth rate were also observed in other plant species, where the presence of blue light was not reported to alter basil plant height or plant FW (Carvalho et al., 2016; Schwend et al., 2016). Leaf area and shoot FW and DW of Chinese broccoli were the highest under blue light proportion at 16%, followed by 24%, then 8%, and the lowest under monochromatic red light at a PPFD of 210 μmol·m^{-2}·s^{-1} with a 12-h photoperiod (He et al., 2015). A possible explanation among these conflicting results could be the interactive responses in plant photosynthesis and photomorphogenesis to blue light. As mentioned above, tested plant species in the present study all had the largest leaf area and greatest shoot FW under treatment with the lowest blue light proportion at 12%, suggesting that the inhibition of plant expansion by blue light dominated in biomass accumulation. However, in Chinese broccoli, a small blue light proportion (16%) may increase biomass accumulation due to blue light increased photosynthetic capacity and a continued increase of blue light

proportion (24%) may eventually lead to the domination of blue light-inhibition of plant expansion growth and result in reduced biomass accumulation (He et al., 2015).

Green light could reverse blue light induced responses such as inhibition of extension growth and evoke shade-avoidance responses, which result in increasing green light interception and biomass accumulation (Folta and Maruhnich, 2007; Zhang et al., 2011). However, higher green light proportions will reduce the photon flux of red and/or blue light, resulting in passive effects on plant growth. For example, leaf area, leaf thickness, and shoot FW and DW in 'Waldmann's Green' lettuce plants increased by $R_{61}B_{15}G_{24}$ treatment (supplemental green fluorescent light to combined R&B LED light) compared to plants grown under combined R&B light with the same blue light proportion ($R_{84}B_{16}$) at a PPFD of 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 18-h photoperiod, while a higher green light proportion at 51% ($R_{30}B_{19}G_{51}$, white fluorescent light) showed no effects on leaf area, shoot FW and DW, and green fluorescent light ($R_4B_{10}G_{86}$) decreased its leaf area, shoot FW and DW (Kim et al., 2004). Similarly, Hernández et al. (2016) reported that a green light proportion at 28% ($R_{52}B_{20}G_{28}$) treatment did not affect plant growth in 'Cumlaude' cucumber seedlings as all growth parameters (leaf mass per area, plant height, and leaf area) followed the trend of other combined R&B treatments ($R_{90}B_{10}$, $R_{70}B_{30}$, $R_{50}B_{50}$, and $R_{25}B_{75}$), although they didn't directly compare it to a $R_{80}B_{20}$ treatment. In the present study, at a low blue light proportion at 12%, additional green light significantly increased plant height in green basil and green mustard plants, while at a higher blue light proportion at 24%, additional green light decreased plant height in purple basil, green kale, red kale, and green mustard plants (Table 8). We postulate that at a low blue light proportion at

12%, green light reverses the blue light inhibition responses and induces stem elongation (shade avoidance response), while at a high blue light proportion at 24%, predominant suppression of expansion growth by blue light may override weaker control of expansion growth by green light and resulted in shorter plant heights. These results were consistent with the results reported by Wang and Folta (2013), in which hypocotyl length in arabidopsis plants increased with additional green light to a low R&B photon flux (< 1-10 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and showed no effects at a high R&B photon flux ($\geq$ 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$). A different pattern was observed in leaf area and shoot FW, where additional green light decreased or tended to decrease (no significant difference) leaf area and shoot FW at both low and high blue light proportions (Tables 8, 9). Kang et al. (2016) also reported that leaf length or width in lettuce plants was not affected by additional green light to combined R&B light with blue light proportions from 0% to 30%. The hypothesis is that green light effects on leaf expansion was minimal compared to its effects on stem elongation. Furthermore, we concluded that leaf area is the major contribution to plant biomass accumulation compared to P_n, since leaf area has the similar trend as shoot FW under different light quality treatments.

3.5.3 Secondary metabolites accumulation as influenced by red, blue, and green light

Although mechanism of light spectrum affecting phytochemical biosynthesis is still unclear, both red and blue lights are believed to be involved in the synthesis of secondary metabolites, and their effects are dependent on plant species and cultivars (Cocetta et al., 2017; Taulavuori et al., 2016). For example, anthocyanin concentration in lettuce, sage

(*Salvia miltiorrhiza*), and kale plants increased under blue light treatment, as well as phenolics concentration in purple basil plants (Hosseini et al., 2018; Li, 2010; Meng et al., 2004; Qian et al., 2016). Similarly, antioxidant capacity in lettuce and kale plants both increased under enriched blue light treatments (Qian et al., 2016; Son and Oh, 2013). Induced synthesis of secondary metabolites by blue light is supported by Li (2010) and Meng et al. (2004), who reported that expression of key enzymes in the synthesis of polyphenols, such as phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), and dihydroflavonol 4-reductase (DFR) increased under blue light. However, in other plant species, blue light played a negative role or showed no effects on the synthesis of secondary metabolites. For instance, monochromatic red light enhanced anthocyanin concentration in red leaf cabbage seedlings (*Brassica oleracea* 'Red Rookie') compared to monochromatic blue or green light, while light quality did not affect anthocyanin concentration in green leaf cabbage seedlings ('Kinshun') at a PPFD of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 16-h photoperiod (Mizuno et al., 2009). Antioxidant capacity of basil plants decreased with increases of blue light proportions from 30% to 58% and showed no differences with increases of blue light proportions from 19% to 30%, while flavonoid concentration was the highest under treatment with blue light proportion at 23%, followed by 30% and 44%, and the lowest under 19% and 58% (Pennisi et al., 2019). Piovene et al. (2015) also reported that antioxidant capacity and concentrations of polyphenol and flavonoids in basil plants was not affected by changes of blue light proportions from 7% to 38% in combined R&B LEDs. In the present study, increases of blue light proportions enriched concentrations of anthocyanin, phenolics, and flavonoids in most species (Table

10), but decreased the total amount phytochemicals per plant (Table 11). Therefore, two mechnisms of increased phytochemical concentrations under higher blue light proportions were postulated. One hypothesis is that higher blue light proportions induced expression of key enzymes in the phenylpropanoid pathway, such as PAL, CHS, and DFR, which induced synthesis of anthocyanin, phenolics, and flavonoids (Li, 2010; Meng et al. 2004). The other hypothesis is the dilution effect caused by red light. Which is, with similar total amount of phytochemicals per plant, plant shoot FW increased and resulted in decreased phytochemical concentrations under higher red light proportions.

The interactions between green and combined R&B light are hard to predict, but are known to be mediated by photosensory pathways, as photoreceptors such as phytochromes, phototropins, and cryptochromes also absorb green light (Folta and Maruhnich, 2007; Wang and Folta, 2013; Zhang and Folta, 2012). Folta and Maruhnich (2007) reported that green light reversed blue light induced anthocyanin accumulation, which was confirmed in arabidopsis and 'Red Sails' lettuce plants (Zhang and Folta, 2012; Zhang et al., 2011). Similarly, additional green light ($R_{29}B_{31}G_{40}$, white FLs) significantly decreased the antioxidant capacity and flavonoid concentration in basil plants compared to plants grown under $R_{62}B_{30}$ treatment at a PPFD of 215 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 16-h photoperiod (Pennisi et al., 2019). However, green light showed both positive and negative effects on the phytochemical accumulation in the present study. Specifically, additional green light decreased concentrations of phenolics, flavonoids and antioxidant capacity in green and purple basil plants regardless of blue light proportion, while in green kale plants,

additional green light increased phenolic concentration and antioxidant capacity at a higher blue light proportion at 24% (Table 10).

3.6 Conclusion

Plant photosynthesis, growth, morphology, and yield in tested plant species were primarily influenced by R&B light than green light. Increases of blue light proportions significantly increased P_n and SPAD readings in purple basil and red kale plants, but negatively influenced plant growth rate or biomass accumulation, indicating blue light inhibition of plant expansion growth dominated in biomass accumulation. Additional green light to combined R&B light played a negative role or had no effects on P_n, SPAD readings, leaf area, or plant yield in tested plant species, and the green light effects on leaf expansion were minimal compared to background R&B light. Effects of red, blue, and green lights on secondary metabolites accumulation are more complicated: increases of blue light proportions played a positive role or had no effects on the synthesis of anthocyanin, phenolics, and flavonoids in tested plant species, while additional green light showed both positive and negative effects on the accumulation of phytonutrients. However, considering plant yield decreased by green light, combined R&B light with lower blue light proportions would be recommended for culinary herbs and leafy greens production in IVFs.

CHAPTER IV

SUBSTITUTING RED AND/OR BLUE LIGHT WITH GREEN LIGHT INDUCED SHADE AVOIDANCE RESPONSES BUT DECREASED PHOTOSYNTHESIS AND SECONDARY MEATBOLITES ACCUMULATION IN BASIL PLANTS

4.1 Synopsis

Green light penetrates into deeper plant canopy due to its high transmittance and reflectance than the other wavelengths, while red and blue lights are mostly absorbed by the upper level leaves. Theoretically, substituting partially red and/or blue light with green light could increase light interception by inner canopy, which could potentially increase plant yield. Therefore, we studied the effects of substituting partial red and/or blue light with green light on plant photosynthesis, growth, and development in basil (*Ocimum basilicum*) 'Improved Genovese Compact' (green) and 'Red Rubin' (purple) plants. There were four treatments including combined red and blue (R&B) light treatment as control, $R_{76}B_{24}$ (the percentage of red and blue light was 76% and 24%, respectively), substituting partial red light with green light, $R_{44}B_{24}G_{32}$, substituting partial blue light with green light, $R_{74}B_{16}G_{10}$, and substituting partial red and blue light with green light, $R_{42}B_{13}G_{45}$. All experiments were conducted in a growth room with the same photosynthetic photon flux density (PPFD) of 224 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 16-h photoperiod. Plants were sub-irrigated as needed using a nutrient solution with electrical conductivity of 2.0 $dS \cdot m^{-1}$ and pH of 6.0. In green basil plants (5 internodes at harvest), net photosynthetic rate of the upper level leaves was the highest under $R_{76}B_{24}$ treatment, while no difference was observed in the

lower level leaves. In purple basil plants (7 internodes at harvest), net photosynthetic rate of the upper level leaves showed no differences among $R_{76}B_{24}$, $R_{44}B_{24}G_{32}$, or $R_{74}B_{16}G_{10}$ treatments, while the highest under $R_{44}B_{24}G_{32}$ and $R_{74}B_{16}G_{10}$ treatments in the lower level leaves. Plant height of both cultivars increased under $R_{44}B_{24}G_{32}$ and $R_{42}B_{13}G_{45}$ treatments. Shoot fresh weight of green basil plants was not affected by green light treatments, while increased under $R_{76}B_{24}$ and $R_{74}B_{16}G_{10}$ treatments in purple basil plants. Nutritional quality of green basil plants, including concentrations of anthocyanin, total phenolics and flavonoid, and antioxidant capacity of plant leaves, increased with increasing blue light proportions ($R_{76}B_{24}$ and $R_{44}B_{24}G_{32}$ treatments), while in purple basil plants, it increased with increasing red light proportions ($R_{76}B_{24}$ and $R_{74}B_{16}G_{10}$ treatments).

4.2 Introduction

Plants sense and respond to a broad range of light spectra from ultraviolet to far-red regions, while photosynthetically active radiation, ranging from 400-700 nm [blue (400-499 nm), green (500-599 nm), red (600-700 nm)], significantly affects plant photosynthesis, morphology, and secondary metabolism (Amaki et al., 2011; Brazaitytė et al., 2016). The development of light emitting diode (LED) technology provided researchers opportunities to regulate plant yield and nutritional quality using different light wavelength, which was proven to be a good tool for plant production in indoor vertical farms (IVFs) (Bantis et al., 2018; Dou et al., 2017; Piovene et al., 2015).

Among all light spectra, red and blue lights are the most important for plant biomass accumulation by affecting plant photosynthesis and photomorphogenesis (Mccree, 1972).

It was reported that dominant red with supplemental blue light reached greater plant yield compared to monochromatic red or blue light for crop production under controlled environment (Bondada and Syvertsen, 2003; Wollaeger and Runkle, 2014). For example, leaf area, shoot fresh weight (FW), and shoot dry weight (DW) in spinach (*Spinacia oleracea*) and non-heading Chinese cabbage (*Brassica campestris* 'Te Ai Qing') increased under combined R&B LED light compared to monochromatic red or blue LED light (Fan et al., 2013; Ohashi-Kaneko et al., 2007). Similarly, leaf area and shoot FW in baily (*Brassica alboglabra*) plants grown under combined R&B light (blue light proportion ranging from 8% to 24%) were 36-121% and 34-119% higher compared to plants grown under monochromatic blue light, respectively (He et al., 2015).

Comparing to red and blue lights, green light is less studied due to its low absorptivity coefficient in the absorption spectra of chlorophylls compared to red or blue lights. However, green light also contributes to plant growth, which can trigger specific and necessary responses of plant growth (Meng et al., 2019). According to Sager et al. (1988), in a living leaf or whole plant canopy, the relative quantum efficiency for broadband green light is 0.87, which is slightly lower than for red light (0.91) but higher than for blue light (0.73). Furthermore, green light penetrates into deeper plant canopy, scatters between cellular components within leaves, while red and blue lights are mostly absorbed by the upper level plant canopy and drives photosynthesis through abundant lower chloroplasts (Meng et al., 2019; Terashima et al., 2009; Wang and Folta, 2013). Theoretically, quantum yield of a dense plant canopy should be more equalized under green light by increasing the light interception of lower level leaves, which could potentially increase plant yield.

In fact, Paradiso et al. (2011) validated that canopy quantum efficiency of green light was not much lower than that of red light in ‘Akito’ rose (*Rosa*) plants. Kim et al. (2004) also reported that substituting partial red light with green light increased leaf area and shoot FW and DW in ‘Waldmann’s Green’ lettuce (*Lactuca sativa*) plants by 31%, 45%, and 47%, respectively, compared to plants grown under combined R&B light. Supplementing green light to continuous R&B light also alleviated the degree of photosynthetic capacity reduction and/or injury in ‘Butterhead’ lettuce plants (Bian et al., 2016; Bian et al., 2018). Compared to the other light spectra, green light was reported to induce disease resistance to strawberry anthracnose (*Glomerella cinglata*) and spider mite in ‘Sachinoka’ strawberry (*Fragaria* × *ananassa*) plants grown in the field (Kudo et al., 2011). Moreover, additional green light to combined R&B light would make plants appear normal green color instead of purplish, which makes visual assessment of physiological disorders easy, and also offer a psychological benefit to farm workers.

It is not surprising that the known photoreceptors such as phytochromes and cryptochromes can respond to green light due to their broad band absorption spectrum that tails into the green light waveband. Banerjee et al. (2007) reported that upon excitation by blue light, the flavin chromophore of cryptochrome is reduced to a semiquinone that can absorb green and yellow lights. Green light could activate phytochrome responses such as seed germination in arabidopsis (*Arabidopsis thaliana*) plants (Wang and Folta, 2013). Consistently, plant responses to green light showed a tendency to counteract blue or red light induced responses, such as inhibition of stem elongation, stomatal opening, or anthocyanin accumulation (Talbott et al., 2006; Zhang and Folta, 2012). For example,

stomatal opening stimulated by blue light could be reversed by green light in a range of plant species (Frechilla et al., 2000; Talbott et al., 2002), and increasing green light proportions significantly decreased anthocyanin concentrations in arabidopsis and 'Red Sails' lettuce plants (Zhang and Folta, 2012; Zhang et al., 2011). Furthermore, plant responses to green light were affected by green light peak wavelength and light intensity (Johkan et al., 2012). Specifically, the biomass accumulation of red leaf lettuce ('Red Fire') showed no differences among three monochromatic green light treatments (G510, G520, and G530, in which the peak wavelength of green light is at 510 nm, 524 nm, and 532 nm, respectively) and white fluorescent light treatment at a PPFD of 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$, while it was the highest under G510 treatment and showed no differences among the other treatments at a PPFD of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Johkan et al., 2012).

Previous studies raise the hypothesis that substituting green light for red and/or blue light may increase plant yield and alter plant secondary metabolites accumulation, and its effects depend on the light intensity or green light proportions. Therefore, in the present study, we partially substituted red and/or blue light with green light at different green light proportions to investigate the effects of green light addition on plant photosynthesis, growth, yield, and secondary metabolites accumulation.

4.3 Materials and Methods

4.3.1 Plant materials and growing conditions

The experiment was conducted in a walk-in growth room in Texas AgriLife Research and Extension Center at El Paso, TX using green basil 'Improved Genovese Compact'

(*Ocimum basilicum*) and purple basil 'Red Rubin' plants (Johnny's Selected Seeds, Winslow, ME, USA). For both cultivars, one seed per cell was sown in 72 square cell trays (cell size: 3.86 cm L × 5.72 cm H, with a volume of 59 cm^3) with Metro-Mix 360 (peat moss 41%, vermiculite 34%, pine bark 25%, Sun Gro® Horticulture, Bellevue, WA, USA). All trays were put under mist in a greenhouse for germination. Seedlings were moved out from mist after germination and grown in a greenhouse for two weeks. Seedlings were then transplanted to 4" square pots (length 9.52 cm, height 8.26 cm; volume 574 cm^3) with Metro-Mix 360 when roots were visible on the outside of the plug root ball, and uniform plants were selected and moved to the walk-in growth room for different treatments.

4.3.2 Green light treatments

There were four different light quality treatments including the combined R&B light treatment as control, $R_{76}B_{24}$ (the percentage of red and blue light was 76% and 24%, respectively; Model GEHL48HPPB, Hort Americas, Bedford, TX, USA), substituting partial red light with green light, $R_{44}B_{24}G_{32}$ (ESW X6, Illumitex, Austin, TX, USA), substituting partial blue light with green light, $R_{74}B_{16}G_{10}$ (ESW F3, Illumitex, Austin, TX, USA), and substituting partial red and blue lights with green light, $R_{42}B_{13}G_{45}$ (Model GEHL48HWTB, Hort Americas, Bedford, TX, USA) (Table 12, Fig. 8). The PPFD of each treatment was set at the same level of 220 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 16-h photoperiod. To minimize light distribution being disproportionate within each treatment, all plants were systematically rearranged every three days. The PPFD in each treatment was measured at

15 cm underneath the light sources at 9 spots using PS-100 spectroradiometer (Apogee Instruments, Logan, UT, USA).

All plants were sub-irrigated with a nutrient solution containing 1.88 $g \cdot L^{-1}$ (277.5 ppm N) 15N-2.2P-12.5K (Peters 15-5-15 Ca-Mg Special, The Scotts Company, Marysville, OH, USA) as needed, maintaining electrical conductivity of 2.0 $dS \cdot m^{-1}$ and pH at 6.0. Plant canopy temperature was recorded and maintained at 24.0/21.6ºC day/night. Mechanical mini fans (LS1225A-X, AC Infinity, City of Industry, CA, USA) were used to circulate the air to achieve uniform temperatures across treatments. All plants were harvested when plant height reached about 25 cm. The green and purple basil plants were harvested at 21 and 28 days after treatment (42 and 53 days after sowing), respectively. There were 12 plants per treatment for each experiment.

Table 12. Light spectrum distribution of different light quality treatments including $R_{76}B_{24}$, $R_{74}B_{16}G_{10}$, $R_{44}B_{24}G_{32}$, and $R_{42}B_{13}G_{45}$ treatments.

	Single-band Photon Flux Density ($\mu mol \cdot m^{-2} \cdot s^{-1}$)			
Treatment	$R_{76}B_{24}$	$R_{44}B_{24}G_{32}$	$R_{74}B_{16}G_{10}$	$R_{42}B_{13}G_{45}$
B	53	54	36	28
G	-	70	22	98
R	169	97	165	93
R:B	3.26	1.81	4.70	3.29
PPFD[z]	222	221	223	219

[z] Photosynthetic photon flux density (PPFD, 400-700 nm) was measured using a PS-100 spectroradiometer.

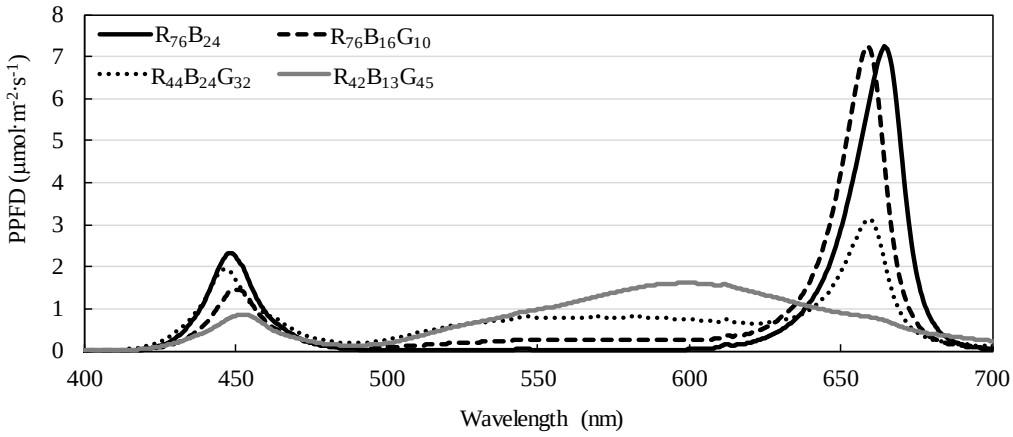

Figure 8. Light spectrum distribution of different light quality treatments including $R_{76}B_{24}$ [in which the red (R, 600-699 nm), blue (B, 400-499 nm), and green (G, 500-599 nm) light percentage is 76%, 24%, and 0%, respectively], $R_{74}B_{16}G_{10}$, $R_{44}B_{24}G_{32}$, and $R_{42}B_{13}G_{45}$ treatments. The photosynthetic photon flux density (PPFD, 400-700 nm) and light spectrum distribution was measured using a PS-100 spectroradiometer.

4.3.3 Measurements

1. Gas exchange and chlorophyll concentration

A portable gas exchange analyzer (CIRAS-3, PP Systems International, Amesbury, MA, USA) was used to measure the gas exchange rate including net photosynthetic rate (P_n), transpiration rate (E), and stomatal conductance (G_s) of plant leaves at harvest. A PLC3 leaf cuvette with LED light unit was used, and light intensity, relative air humidity, and CO_2 concentration inside the leaf chamber were kept constant at 800 μmol·m^{-2}·s^{-1}, 50%, and 390 μmol·mol^{-1}, respectively. The third and fifth pair of leaves from the top were used for measuring the upper and lower leaves gas exchange rate, respectively, in both green and purple basil leaves. Soil plant analysis development (SPAD) index of basil was measured using the third pair of leaves at harvest to quantify relative chlorophyll

content in basil leaves using a chlorophyll meter SPAD-502 (Konica-Minolta cooperation, Ltd., Osaka, Japan).

2. Growth characteristics

Growth characteristics such as plant height, two perpendicular widths, and number of internodes were recorded at harvest. Five plants per treatment were randomly selected for measurement. Leaf area was measured using a leaf area meter (LI-3100, LI-COR, Lincoln, NE, USA), and shoot and root FW were recorded at harvest. Shoot and root tissues were dried at 80ºC in a drying oven (Grieve, Round Lake, IL, USA) for 3 days to determine the shoot and root DW.

3. Secondary metabolites

Five uniform plants were randomly selected for the measurements of concentrations of anthocyanin, phenolics, and flavonoids, and antioxidant capacity at harvest. Fresh plant leaves were collected in a cooler and immediately stored in a deep freezer (IU1786A, Thermo Fisher Scientific, Marietta, OH, USA) at -80ºC until phytochemical analyses.

Extraction. Approximately 2 g fresh plant leaves were ground in liquid nitrogen and extracted with 15 mL 1% acidified methanol at 4ºC in darkness. After overnight extraction, the mixture was centrifuged (Sorvall RC 6 Plus Centrifuge, Thermo Fisher Scientific, Madison, WI, USA) at 13,200 rpm (26,669 ×*g*) for 15 min, and the supernatant was collected for phytochemical analyses (Xu and Mou, 2016).

Anthocyanin analysis. The absorbance of extracts was measured at 530 nm using a spectrophotometer (Genesys 10S ultraviolet/ Vis, Thermo Fisher Scientific, Madison, WI,

USA), and the anthocyanin concentration was expressed as mg cyanidin-3-glucoside equivalent per 100 g FW of basil leaves using a molar extinction coefficient of 29,600 (Connor et al., 2002). Since the extracts were freshly prepared from leaf tissues maintained at -80ºC and did not undergo extensive processing or significant browning, a pH differential method for anthocyanin content was considered unnecessary (Connor et al., 2002).

Phenolics analysis. The total phenolics concentration of plant leaves was determined using the modified Folin-Ciocalteu reagent method described as the following: 100 µL extraction sample was added to a mixture of 150 µL distilled water and 750 µL 1/10 dilution Folin-Ciocalteu reagent. After 6 min reaction, 600 µL 7.5% Na_2CO_3 was added and the mixture was incubated at 45°C in water bath for 10 min before the absorbance was measured at 725 nm using a microplate reader (ELx800, BioTek, Winooski, VT, USA). Results were expressed as mg of gallic acid equivalent per g FW of basil leaves (Xu and Mou, 2016).

Flavonoids analysis. The total flavonoid concentration of plant leaves was determined as the following: 20 µL extraction sample was added to a mixture of 85 µL distilled water and 5 µL 5% $NaNO_2$. After 6 min reaction, 10 µL of 10% $AlCl_3 \cdot 6H_2O$ was added to the mixture. After another 5 min reaction, 35 µL of 1M NaOH and 20 µL distilled water were added to the mixture and the absorbance was measured at 520 nm using the aforementioned microplate reader (Dou et al., 2018). Results were expressed as mg of (+)-catechin hydrate equivalent per g FW of basil leaves.

Antioxidant capacity analysis. The antioxidant capacity of plant leaves was measured using the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) method (Arnao et al., 2001) described as the following: add a mixture of 150 μL basil leave extracts to 2.85 mL of $ABTS^+$ solution and incubate at room temperature for 10 min. The absorbance of mixed solution was measured at 734 nm using the spectrophotometer mentioned above. Antioxidant capacity of basil leaves was expressed as mg of Trolox equivalent antioxidant capacity per 100 g FW of basil leaves.

4.3.4 Statistical analysis

One-way analysis of variance (ANOVA) was conducted to analyze the effects of light quality treatments on all measured parameters. Mean comparison among treatments was conducted using Student's t method. Correlation test was conducted using Pairwise Correlations method. All statistical analyses were performed using JMP software (Version 13, SAS Institute Inc., Cary, NC, USA).

4.4 Results

4.4.1 Photosynthesis and chlorophyll content

In green basil plants, P_n of the upper leaves was the highest under combined R&B light treatment, namely $R_{76}B_{24}$, while it showed no differences among treatments in the lower leaves (Fig. 9A). In contrast, E and G_s in green basil plants showed no differences among treatments regardless of the measuring position (Fig. 9B-C). In purple basil plants, P_n, E and G_s of the upper leaves showed a similar trend, which increased under treatments with

lower green light proportions, namely $R_{76}B_{24}$, $R_{44}B_{24}G_{32}$, and $R_{74}B_{16}G_{10}$, and the lowest under $R_{42}B_{13}G_{45}$ treatment (Fig. 9A-C). In contrast, P_n of the lower leaves increased under $R_{44}B_{24}G_{32}$ and $R_{74}B_{16}G_{10}$, compared to $R_{76}B_{24}$ or $R_{42}B_{13}G_{45}$ (Fig. 9A), whereas E and G_s was the highest under $R_{44}B_{24}G_{32}$, followed by $R_{74}B_{16}G_{10}$ and $R_{76}B_{24}$, and the lowest under $R_{42}B_{13}G_{45}$ (Fig. 9B-C). SPAD readings in green basil plants showed no differences among treatments, while in purple basil plants, it was the highest under $R_{76}B_{24}$, followed by $R_{44}B_{24}G_{32}$ and $R_{74}B_{16}G_{10}$, and the lowest under $R_{42}B_{13}G_{45}$ (Fig. 10).

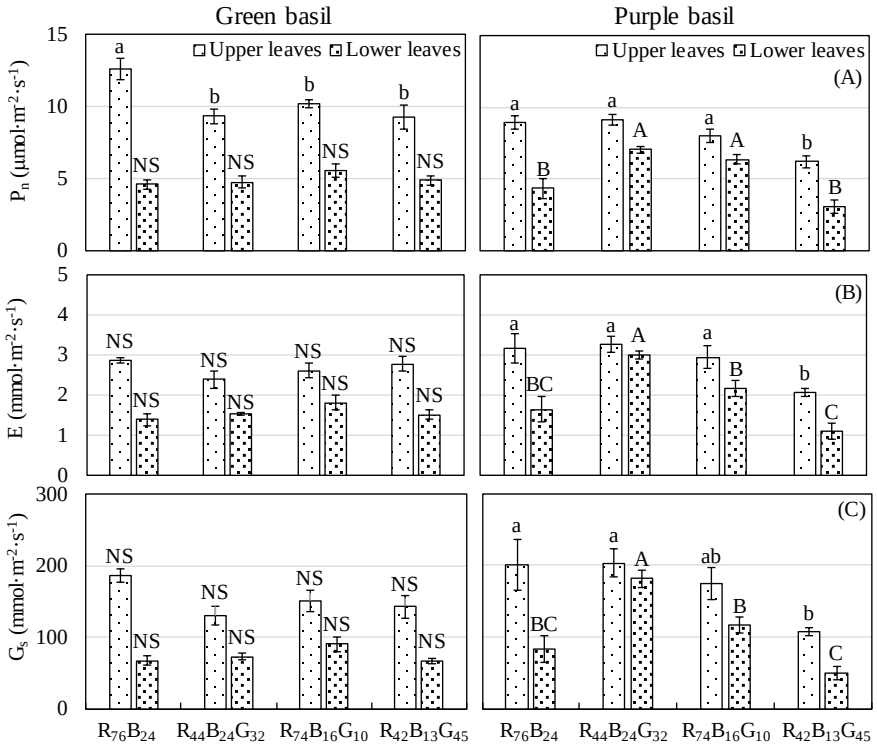

Figure 9. Net photosynthetic rate (P_n) (A), transpiration rate (E) (B), and stomatal conductance (G_s) (C) of green basil 'Improved Genovese Compact' and purple basil 'Red Rubin' plants under different light quality treatments, including $R_{76}B_{24}$, $R_{44}B_{24}G_{32}$, $R_{74}B_{16}G_{10}$, and $R_{42}B_{13}G_{45}$. Means followed by the same lower/upper case letters are not significantly different, according to Student's *t* mean comparison ($P < 0.05$). Bars represent standard errors.

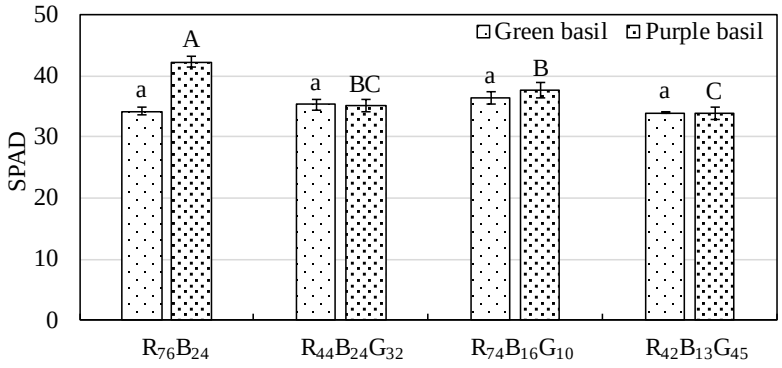

Figure 10. Soil plant analysis development (SPAD) of green basil 'Improved Genovese Compact' and purple basil 'Red Rubin' plants under different light quality treatments, including $R_{76}B_{24}$, $R_{44}B_{24}G_{32}$, $R_{74}B_{16}G_{10}$, and $R_{42}B_{13}G_{45}$. Means followed by the same lower/upper case letters are not significantly different, according to Student's *t* mean comparison ($P < 0.05$). Bars represent standard errors.

4.4.2 Plant growth and yield

Plant height in green and purple basil plants both increased under treatments with higher green light proportions, $R_{44}B_{24}G_{32}$ and $R_{42}B_{13}G_{45}$ compared to $R_{76}B_{24}$ and $R_{74}B_{16}G_{10}$ (Fig. 11A). Plant width (Fig. 11B), leaf area (data not shown), and leaf thickness (Fig. 113C) showed no differences among treatments in green basil plants. In purple basil plants, plant width increased significantly under treatment with the highest green light proportion, $R_{42}B_{13}G_{45}$, which was 15%-18% greater compared to the other treatments (Fig. 11B). Leaf thickness in purple basil plants was the highest under $R_{76}B_{24}$, followed by $R_{74}B_{16}G_{10}$, and the lowest under treatments with higher green light proportions, $R_{44}B_{24}G_{32}$ and $R_{42}B_{13}G_{45}$ (Fig. 11C), while leaf area was not influenced by light quality treatments (data not shown).

Plant yield (i.e., shoot FW and DW, root FW and DW) in green basil plants showed no differences among treatments (Fig. 12A). In purple basil plants, shoot and root FW and DW showed a similar trend, which was higher under treatments with no green light or lower green light proportions, $R_{76}B_{24}$ and $R_{74}B_{16}G_{10}$ compared to $R_{44}B_{24}G_{32}$ and $R_{42}B_{13}G_{45}$ (Fig. 12A-B). Specifically, shoot FW and root FW under $R_{74}B_{16}G_{10}$ was 30% and 88% greater compared to plants grown under $R_{42}B_{13}G_{45}$, respectively. Shoot FW was significantly correlated to leaf area in purple basil plants, while it was not correlated in green basil plants (Fig. 13A). Shoot FW was not correlated to leaf thickness regardless of cultivar (Fig. 13B).

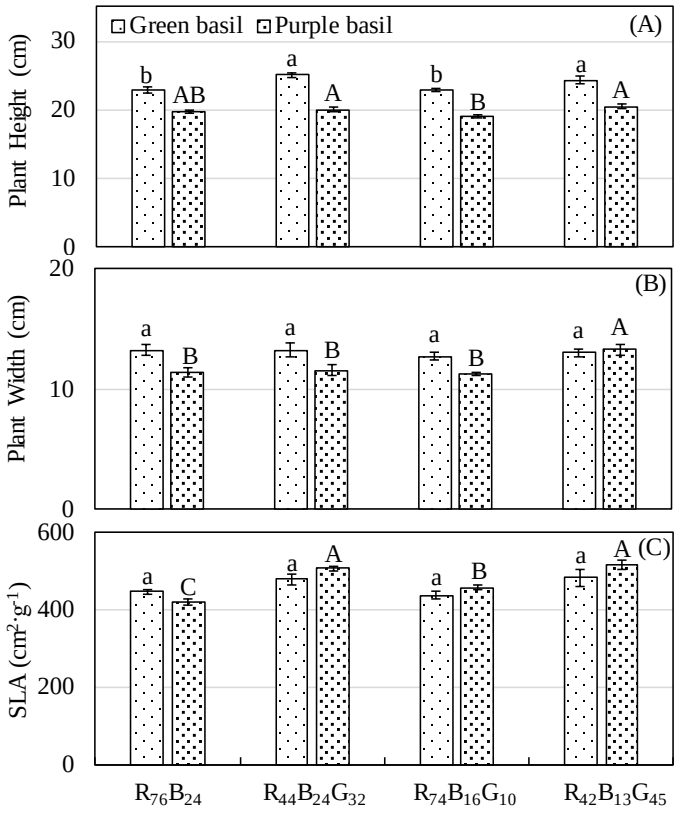

Figure 11. Plant height (A), plant width (B), and specific leaf area (SLA) (C) of green basil 'Improved Genovese Compact' and purple basil 'Red Rubin' plants under different light quality treatments, including $R_{76}B_{24}$, $R_{44}B_{24}G_{32}$, $R_{74}B_{16}G_{10}$, and $R_{42}B_{13}G_{45}$. Means followed by the same lower/upper case letters are not significantly different, according to Student's *t* mean comparison ($P < 0.05$). Bars represent standard errors.

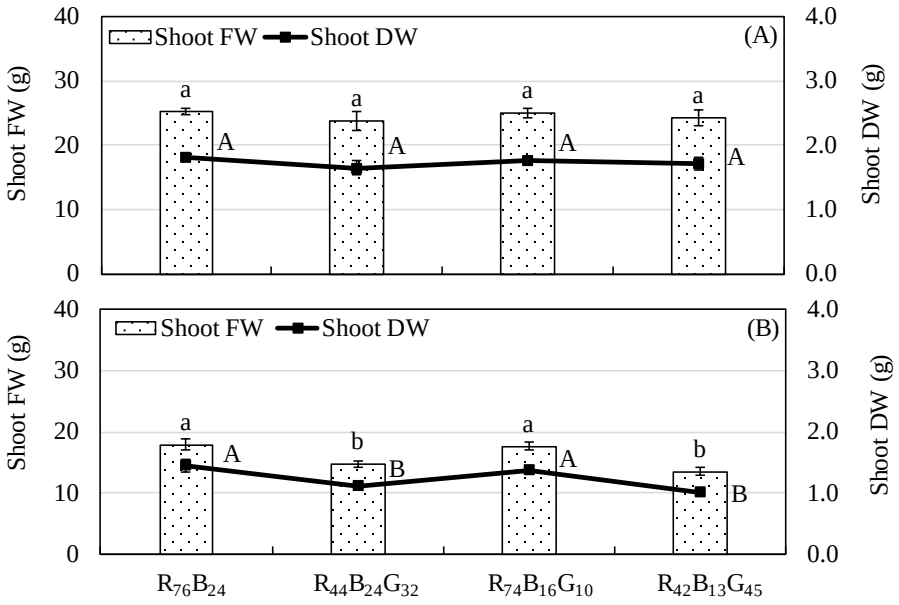

Figure 12. Shoot fresh weight (FW) and dry weight (DW) of green basil plants (A) and purple basil 'Red Rubin' plants (B) under different light quality treatments, including $R_{76}B_{24}$, $R_{44}B_{24}G_{32}$, $R_{74}B_{16}G_{10}$, and $R_{42}B_{13}G_{45}$. Means followed by the same lower/upper case letters are not significantly different, according to Student's *t* mean comparison ($P < 0.05$). Bars represent standard errors.

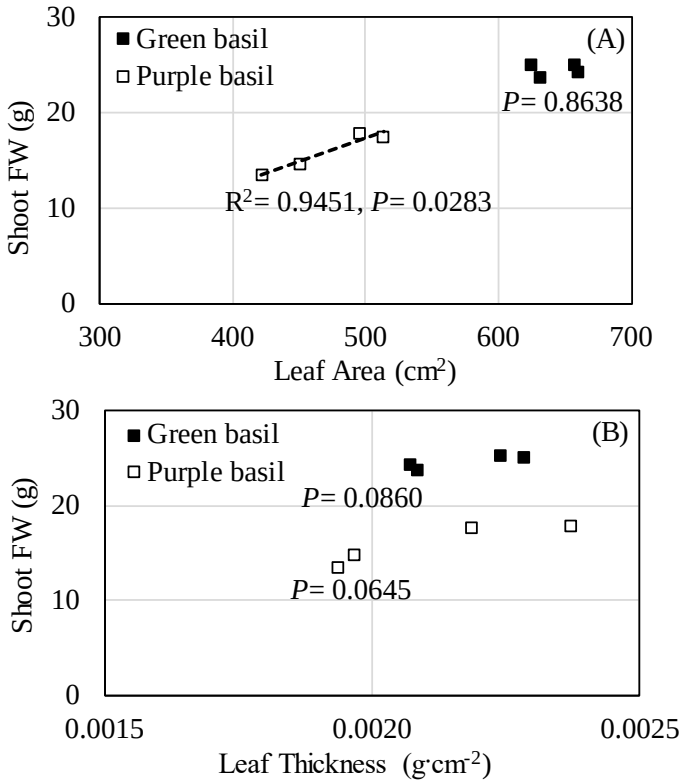

Figure 13. Correlation between shoot FW and leaf area (A), and correlation between shoot FW and leaf thickness (B) in green basil 'Improved Genovese Compact' and purple basil 'Red Rubin' plants under different light quality treatments. Dash line shows the regression between shoot FW and leaf area, according to Pairwise Correlation method.

4.4.3 Accumulation of secondary metabolites

In green basil plants, concentrations of anthocyanin, phenolics, and flavonoids and antioxidant capacity all decreased under $R_{74}B_{16}G_{10}$ and $R_{42}B_{13}G_{45}$ (Table 13). Specifically, concentrations of anthocyanin, phenolics, and flavonoids and antioxidant capacity in green basil plants grown under $R_{76}B_{24}$ was 17%, 18%, 15%, and 20% greater compared to plants grown under $R_{42}B_{13}G_{45}$, respectively. However, in purple basil plants, concentrations of phenolics and flavonoids and antioxidant capacity all decreased under $R_{44}B_{24}G_{32}$ and

$R_{42}B_{13}G_{45}$, while anthocyanin concentration was not influenced by the light quality treatments (Table 13).

Green light treatments decreased the total amount of phytochemicals by 17%-21% in green basil plants (Table 14). In purple basil plants, the total amount of anthocyanin, phenolics, and flavonoids were the highest under $R_{76}B_{24}$ and $R_{74}B_{16}G_{10}$ treatments, followed by $R_{44}B_{24}G_{32}$ treatment, and the lowest under $R_{42}B_{13}G_{45}$ treatment (Table 14). The total amount of antioxidant capacity per plant was the highest under $R_{74}B_{16}G_{10}$ treatment, which was 5%, 41%, and 63% higher compared to $R_{76}B_{24}$, $R_{44}B_{24}G_{32}$, and $R_{42}B_{13}G_{45}$ treatments, respectively (Table 14).

Table 13. Anthocyanin concentration (conc.), phenolics conc., flavonoids conc., and antioxidant capacity of green basil 'Improved Genovese Compact' and purple basil 'Red Rubin' plants under different light quality treatments, including $R_{76}B_{24}$, $R_{44}B_{24}G_{32}$, $R_{74}B_{16}G_{10}$, and $R_{42}B_{13}G_{45}$.

Cultivar	Treatments	Anthocyanin Conc. ($mg \cdot 100g^{-1}$)		Phenolics Conc. ($mg \cdot g^{-1}$)		Flavonoids Conc. ($mg \cdot g^{-1}$)		Antioxidant Capacity ($mg \cdot 100g^{-1}$)	
Green basil	$R_{76}B_{24}$	8.11	a[z]	2.06	a	1.77	ab	366	a
	$R_{44}B_{24}G_{32}$	8.23	a	1.89	ab	1.84	a	353	a
	$R_{74}B_{16}G_{10}$	7.58	ab	1.75	b	1.67	bc	319	b
	$R_{42}B_{13}G_{45}$	6.95	b	1.75	b	1.54	c	306	b
Purple basil	$R_{76}B_{24}$	13.36	A	3.71	A	2.29	AB	1293	A
	$R_{44}B_{24}G_{32}$	14.15	A	3.46	B	2.16	B	1174	B
	$R_{74}B_{16}G_{10}$	13.33	A	3.81	A	2.34	A	1367	A
	$R_{42}B_{13}G_{45}$	12.92	A	2.94	C	1.82	C	1101	B

[z] Means followed by the same lower/upper case letters are not significantly different, according to Student's *t* mean comparison ($P < 0.05$).

Table 14. Total amount of anthocyanin, phenolics, flavonoids, and antioxidant capacity per plant of green basil 'Improved Genovese Compact' and purple basil 'Red Rubin' plants under different light quality treatments, including $R_{76}B_{24}$, $R_{44}B_{24}G_{32}$, $R_{74}B_{16}G_{10}$, and $R_{42}B_{13}G_{45}$.

Cultivar	Treatments	Total Amount of Phytochemicals (mg·plant^{-1})							
		Anthocyanin		Phenolics		Flavonoids		Antioxidant Capacity	
Green basil	$R_{76}B_{24}$	1.63	a[z]	41	a	35	a	73	a
	$R_{44}B_{24}G_{32}$	1.54	a	35	b	34	a	66	b
	$R_{74}B_{16}G_{10}$	1.51	a	35	b	33	a	64	b
	$R_{42}B_{13}G_{45}$	1.32	b	33	b	29	b	58	c
Purple basil	$R_{76}B_{24}$	1.90	A	53	A	33	A	184	B
	$R_{44}B_{24}G_{32}$	1.66	B	41	B	25	B	138	C
	$R_{74}B_{16}G_{10}$	1.90	A	54	A	33	A	194	A
	$R_{42}B_{13}G_{45}$	1.40	C	32	C	20	C	119	D

[z] Means followed by the same lower/upper case letters are not significantly different, according to Student's *t* mean comparison ($P < 0.05$).

4.5 Discussion

4.5.1 Substituting red or blue light with green light increased photosynthesis in the lower level plant canopy

Additional green light repressed or had no effects on plant photosynthesis due to low absorption of green light by chlorophylls (Folta and Maruhnich, 2007; Mccree, 1972; Wang and Folta, 2013), which was confirmed in this study where P_n in the upper leaves decreased in both cultivars (Fig. 9A). It was evidenced that decreased P_n by green light was contributed to, or at least partially contributed to, green light reversed blue light induced stomatal opening and chloroplast synthesis, which was found in a diversity of plant species (Talbott et al., 2006; Talbott et al., 2002). Consistently, in purple basil plants, E and G_s of the upper leaves decreased under $R_{42}B_{13}G_{45}$ treatment, while SPAD readings

decreased under all green light treatments, but the depression by green light was not observed in green basil plants (Fig. 9B-C, 10). These phenomena suggested that low absorption of green light by chlorophylls is the major reason of decreased P_n in green basil plants, while in purple basil plants, decreased P_n might be caused by the coactions of reduced stomatal opening, decreased chloroplast accumulation, and low absorption of green light by chloroplasts.

Red and blue lights were strongly absorbed on the upper level plant canopy, but green light, which is hard for chloroplasts to absorb, penetrated and was absorbed by the chloroplasts in the lower level plant canopy (Terashima et al., 2009). This resulted in increased PPFD in lower level plant canopy under green light treatments compared to combined R&B light treatment, and accordingly a different pattern between P_n in the lower leaves and upper leaves was observed. Specifically, in green basil plants, P_n in the lower leaves was not influenced by light quality treatments while it was decreased under green light treatments in the upper leaves, and in purple basil plants, P_n in the lower leaves increased under green light treatments with green light proportions at 10% and 32% while it showed no differences in the upper leaves (Fig. 9A). Therefore, increased PPFD in the lower level plant canopy by green light may potentially increase the photosynthetic productivity of whole plant canopy, which may result in greater plant yield.

Plant photosynthetic responses to green light depend on plant species and green light intensity or green light proportion. Specifically, P_n in purple basil plants only decreased under treatment with the highest green light proportion of 45%, whereas P_n in green basil plants decreased under all green light treatments (Fig. 9A). The different responses

between green and purple basil plants may be due to their different sensitivity to green light. Another hypothesis is due to their different plant canopy density. Although both cultivars were harvested at similar plant height, green basil plants were treated for 3 weeks and harvested with 5 internodes, while purple basil plants were treated for 4 weeks and harvested with 7 internodes. The denser plant canopy in purple basil plants strengthened the effects of green light since green light penetrates deeper in dense plant canopy. Other researchers also reported different plant responses to green light treatments. For instance, substituting green light for red light decreased P_n and chlorophyll concentration in 'Green Skirt' lettuce plants compared to combined R&B light treatment, while P_n in 'Waldmann's Green' lettuce was not affected (Kang et al., 2016; Kim et al., 2004). However, none of the studies evaluated the relationship between effects of green light and plant canopy density, which should be paid attention to in future studies.

4.5.2 Substituting green light for red and/or blue light induced shade avoidance responses

It has been widely reported that green light could induce shade avoidance responses including promotion of petiole elongation and hyponasty in arabidopsis plants (Folta and Maruhnich, 2007; Zhang et al., 2011). Shade avoidance responses induced by green light are likely mediated in two categories, cryptochrome-dependent and cryptochrome-independent pathways, suggesting an unknown green light receptor through a novel mechanism (Folta, 2004; Wang and Folta, 2013). It was evidenced in the present study when substituting green light for red or red and blue light resulted in increased plant height

and decreased leaf thickness (Fig. 12A). Meanwhile, plant width in purple basil plants increased under treatments of substituting red and blue light with green light, but not leaf area, indicating green light treatment increased petiole elongation but not leaf expansion. Similarly, Meng et al. (2019) reported that substituting blue light with green light increased petiole length in kale (*Brassica oleracea*) plants at a PPFD of 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$. However, biomass accumulation in purple basil plants decreased under treatments substituting red or red and blue light with green light, which was different from the results reported by Meng et al. (2019), in which green light induced shade avoidance responses in kale and lettuce plants and resulted in greater shoot FW. Differences between the present study and Meng's et al. (2019) study might be due to different plant canopy architecture or density (e.g. leaf area index) among lettuce, kale and basil plants. Lettuce and kale plants are almost stemless and have rosette-like ground leaves during vegetative stage (when grown as vegetable crop, and not seed crop), while basil plants have stems, and the compactness of plant canopy of lettuce and kale plants would strengthen the effects of green light, which increases plant shade avoidance responses and thus increases light interception, resulting in greater biomass accumulation. Similarly, green light treatment increased leaf area, leaf thickness, and shoot FW and DW in 'Waldmann's Green' lettuce plants (Kim et al., 2004), but did not affect the growth of 'Cumlaude' cucumber (*Cucumis sativus*) seedlings (Hernández et al., 2016), which has a different plant canopy architecture/density from lettuce plants.

4.5.3 Substituting green light for red and/or blue light decreased secondary metabolites accumulation

Although mechanisms of how light quality affects plant secondary metabolism is still unclear, shared facts were evidenced that green light could reverse the red and blue light induced phytochemical accumulation through photoreceptor pathways (Zhang and Folta, 2012; Zhang et al., 2011). In chapter III, substituting green light for red light (green light proportions of 44% and 41%) decreased phenolic and flavonoid concentrations and antioxidant capacity in both green and purple basil plants regardless of blue light proportions (12% and 24%). Similar results were reported by Pennisi et al. (2019), in which substituting green light for red light significantly decreased antioxidant capacity and flavonoid concentration in basil plants. In the present study, substituting green light for red and/or blue light decreased both secondary metabolites concentrations and total amounts of phytochemicals per plant in both basil cultivars. Noticeably, substituting blue light with green light decreased phytonutrients accumulation and antioxidant capacity in green basil plants, while substituting red light with green light decreased phytonutrients concentration and antioxidant capacity in purple basil plants (Table 13). Therefore, it was postulated that blue and red light plays a major function in inducing the secondary metabolites accumulation in green and purple basil plants, respectively.

4.6 Conclusion

Green and purple basil plants showed different sensitivity to red, blue, and green light regarding to photosynthesis, morphology, and secondary metabolites accumulation. In

general, substituting red and/or blue light with green light decreased plant photosynthesis in the upper level plant canopy, while increased photosynthesis in the lower level plant canopy due to increased PPFD. Meanwhile, substituting red and/or blue light with green light induced plant shade avoidance responses (such as stem and petiole elongation) and decreased secondary metabolites accumulation, but did not influence leaf expansion. In conclusion, substituting red and/or blue light with green light decreased plant yield and phytochemical production in basil plants, which has a relatively low plant canopy density.

CHAPTER V

RESPONSES OF PHOTOSYNTHESIS, GROWTH, AND SECONDARY METABOLITES ACCUMULATION IN BASIL, KALE, AND MUSTARD PLANTS TO PRE-HARVEST UV-B RADIATION AND PHOTOSYNTHETIC PHOTON FLUX DENSITY

5.1 Synopsis

Supplemental ultraviolet-B (UV-B) radiation and photosynthetic photon flux density (PPFD) are both important environmental factors that influence plant photosynthesis, growth, yield, and the accumulation of secondary metabolites, and interaction effects were observed between supplemental UV-B radiation and PPFD. Two experiments were conducted to investigate the responses of basil (*Ocimum basilicum*) and *Brassica* plants to supplemental UV-B radiation and PPFD. In the first experiment, green basil 'Improved Genovese Compact' and purple basil 'Red Rubin' plants grown at two PPFDs, 160 and 224 $\mu mol \cdot m^{-2} \cdot s^{-1}$, were treated with supplemental UV-B radiation at five pre-harvest doses including control (no UV-B), 1 $h \cdot d^{-1}$ for 2 days, 2 $h \cdot d^{-1}$ for 2 days, 1 $h \cdot d^{-1}$ for 5 days, and 2 $h \cdot d^{-1}$ for 5 days. In the second experiment, four *Brassica* plant species were exposed to five UV-B radiation doses including control, 0.5 $h \cdot d^{-1}$ for 1 day (0.5H1D), 1 $h \cdot d^{-1}$ for 1 day (1H1D), 1 $h \cdot d^{-1}$ for 2 days (1H2D), or 1 $h \cdot d^{-1}$ for 3 days (1H3D). Results indicated that plant growth and yield of both basil cultivars decreased under all UV-B treatments, while increased by high PPFD despite UV-B radiation doses. Shoot fresh weight in green and purple basil plants decreased under UV-B treatments by 12-51% and 6-44%,

respectively. Although UV-B depressed plant photosynthesis in *Brassica* plants, it showed no effects on plant growth or yield. Concentrations of anthocyanin, phenolics, and flavonoids in green basil leaves increased under all UV-B treatments by 9-18%, 28-126%, and 80-169%, respectively, and the magnitude of increase was greater under low PPFD compared to high PPFD. Antioxidant capacity in green kale, red kale, and green mustard plants increased under 1H2D and 1H3D treatments. However, in purple basil plants, UV-B radiation showed no effects on anthocyanin concentration, while 2 $h \cdot d^{-1}$ for 2 days and 5 days UV-B treatments increased concentrations of phenolics and flavonoids. Among all treatments, a pre-harvest UV-B radiation of 1 $h \cdot d^{-1}$ for 2-3 days with PPFD of 224 $\mu mol \cdot m^{-2} \cdot s^{-1}$ enriched plant secondary metabolite accumulation without reducing biomass accumulation, which was recommended for green basil and *Brassica* plants production under controlled environment.

5.2 Introduction

Ultraviolet radiation is an important environmental signal that initiates plant responses in photosynthesis, cell division, plant growth, and development (Goto et al., 2016; Wargent, 2016). In previous studies, UV-B radiation was mainly considered as a stress factor to plants, focusing on the effects of increasing solar UV-B radiation reaching earth's surface due to stratospheric ozone depletion (Caldwell and Flint, 1994; Wargent et al., 2009). Recent studies have highlighted supplemental UV-B radiation as "positive stress" which induces a range of beneficial processes in plants including DNA repair, antioxidant induction, and increased synthesis of UV-absorbing compounds, such as phenolics,

flavonoids, carotenoids, and glucosinolates (Sun et al., 2012; Castagna et al., 2014; Moreira-Rodríguez et al., 2017). Epidemiological studies suggested that diets high in antioxidants, especially polyphenolic compounds such as flavonoids and phenolic acids, can reduce the risks of cardiovascular and chronic diseases (Schreiner et al., 2012). This has drawn a lot of attention on using supplemental UV-B radiation as a tool to increase concentrations of these phytonutrients in horticultural crops (Connor et al., 2002; Colonna et al., 2016; Henry-Kirk et al., 2018).

Studies to date have demonstrated at least two UV-B signaling pathways are determined by UV-B radiation doses (Schreiner et al., 2012; Dotto and Casati, 2017). Firstly, the UV-B specific photoreceptor, UV RESISTANCE LOCUS 8 (UVR8), initiates UV-B mediated signaling pathways in response to low UV-B radiation dose, the UVR8-dependent pathway (Henry-Kirk et al., 2018). Under low UV-B radiation doses, UVR8 stimulates the expression of genes such as CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1), ENLONGATED HYPOCOTYL 5 (HY5), and HY5 HOMOLOG (HYH), which play key roles in the synthesis of phenolic compounds, as well as growth retardation such as the inhibition of hypocotyl elongation (Jansen and Bornman, 2012; Holl et al., 2018). Secondly, high UV-B radiation doses induce damage responses in plants, through UVR8-independent pathway (Brown and Jenkins, 2008; Dotto and Casati, 2017). High UV-B radiation doses induce the formation of reactive oxygen species (ROS), causing damage to plant cells, DNA, proteins, and photosynthesis apparatus, and subsequently affect plant growth and development (Brown and Jenkins, 2008; Favory et al., 2009).

In addition to being dose-dependent responses, plant responses to supplemental UV radiation also varied among species and cultivars (Suchar and Robberecht, 2018). For example, anthocyanin concentration of red leaf lettuce (*Lactuca sativa* 'Red Cross') increased by 11% after 12-days UV-A radiation at 18 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 16 $h \cdot d^{-1}$ prior to harvest (controlled environment, PPFD of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (Li and Kubota, 2009). Another study reported that synthesis of anthocyanins and other polyphenols in a different red leaf lettuce cultivar ('Red Fire', controlled environment, PPFD of 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$) increased significantly after 3-days UV-B radiation at 1.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 16 $h \cdot d^{-1}$ prior to harvest (Goto et al., 2016). In 7-day-old broccoli (*Brassica oleracea*) sprouts (controlled environment, PPFD not mentioned), glucosinolate concentration was enhanced by 19% after 1-day UV-B radiation at 7.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 2 $h \cdot d^{-1}$, compared to 63% enhancement at 10.3 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 2 $h \cdot d^{-1}$ (Moreira-Rodríguez et al., 2017). Therefore, the use of supplemental UV-B radiation to increase concentrations of secondary plant metabolites requires "precise manipulation" with respect to different plant species or cultivars.

Basil and *Brassica* plants are highly diverse in species and cultivars and are a valuable part of human diet owing to their relatively high levels of bioactive secondary metabolites (Keservani et al., 2010; Makri and Kintzios, 2008; Qian et al., 2016). For stable and reliable supplies of culinary herbs and leafy greens, more growers are adopting to controlled environment production, especially indoor vertical farms (IVFs), which has been proven to be a suitable alternative to open field and greenhouse production (Liaros

et al., 2016). However, crops cultivated in IVFs are not exposed to UV-B radiation, which is known to influence the accumulation of bioactive phenolic compounds. For this reason, there is increasing interest in the use of supplemental UV-B radiation in IVFs, which allow for year-round production of horticultural crops with high value bioactive compounds (Hogewoning et al., 2012; Stutte, 2016; Wargent, 2016). Although some studies have investigated the effects of UV-B radiation on secondary metabolites accumulation in plants, most were conducted in open field or greenhouse using color filters, and results varied tremendously in both biomass production and phenolic contents (Johnson et al., 1999; Sakalauskaite et al., 2012, 2013). Furthermore, most studies only focused on the effects of UV-B radiation on secondary metabolites accumulation or yield, instead of studying on both bioactive compound accumulation and yield systematically (Johnson et al., 1999; Mosadegh et al., 2018).

Plants response to UV-B radiation is dependent on other environmental factors such as the PPFD (Schreiner et al., 2012). For example, Behn et al. (2010) reported that UV-B radiation can compensate for the reduced accumulation of monoterpene concentration in peppermint (*Mentha x piperita*) leaves (controlled environment) grown under low PPFD (550 $\mu mol \cdot m^{-2} \cdot s^{-1}$) compared to high PPFD (1,150 $\mu mol \cdot m^{-2} \cdot s^{-1}$). However, after being treated with UV-B radiation of 0.65 $kJ \cdot m^{-2} \cdot h^{-1}$ (controlled environment), concentration of quercetin derivatives in arabidopsis (*Arabidopsis thaliana*) plants was significantly greater under high PPFD (1,310 $\mu mol \cdot m^{-2} \cdot s^{-1}$) compared to low PPFD (540 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (Götz et al., 2010). In our previous study, PPFD was positively correlated with the phytonutrient concentration including anthocyanin, phenolics, and flavonoids in green basil ‘Improved

Genovese Compact' plants (Dou et al., 2018). However, little information is known about the interactive effects between supplemental UV-B radiation and PPFD on the growth and accumulation of phenolic compounds in basil plants grown under controlled environment with artificial lighting.

In this study, we conducted two experiments to investigate the optimal UV-B radiation dose and its combination with different PPFD levels. In the first experiment, we exposed two cultivars of basil plants to five pre-harvest supplemental UV-B radiation doses to investigate the responses of basil plants to UV-B radiation at two PPFDs in IVFs. Photosynthetic photon flux density of 224 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was selected for basil plants based on our previous study (Dou et al., 2018), and a lower PPFD, 160 $\mu mol \cdot m^{-2} \cdot s^{-1}$, was selected to investigate if UV-B radiation can compensate for the reduced accumulation of phytonutrients in basil plants grown under low PPFD. In the second experiment, we investigated responses of four *Brassica* plant species to five pre-harvest supplemental UV-B radiation doses. Accordingly, an optimal UV-B radiation dose and PPFD level was determined to achieve enhanced accumulation of secondary plant metabolites in basil and *Brassica* plants without significant yield reduction.

5.3 Materials and Methods

5.3.1 Plant materials and growing conditions

Two experiments were conducted in a walk-in growth room in Texas AgriLife Research Center at El Paso, TX. The first experiment was conducted to investigate the

interactive effects between pre-harvest supplemental UV-B radiation and PPFD on basil (*Ocimum basilicum*) 'Improved Genovese Compact' (green) and 'Red Rubin' (purple) (Johnny's Selected Seeds, Winslow, ME, USA). The second experiment was conducted to investigate different pre-harvest supplemental UV-B radiation doses on the growth and nutritional quality in green kale 'Siberian' (*Brassica napus pabularia*), red kale 'Scarlet' (*Brassica oleracea*), green mustard 'Amara' (*Brassica carinata*), and red mustard 'Red Giant' (*Brassica juncea*) plants (Johnny's Selected Seeds, Winslow, ME, USA). In both experiments, one seed per cell was sown in 72 square cell trays (length 3.86 cm; height 5.72 cm; volume 59 cm^3) with Metro-Mix® 360 (peat moss 41%, vermiculite 34%, pine bark 25%, Sun Gro® Horticulture, Bellevue, WA, USA). All trays were put under mist in a greenhouse for germination. The temperature under the mist was maintained at 32.7ºC/22.2ºC day/night. Seedlings were moved out from the mist after emergence of cotyledons and grown in a greenhouse for two weeks. The temperature and relative humidity in the greenhouse were maintained at 29.1ºC/21.6ºC and 48%/66% day/night, respectively. When one pair of fully expanded true leaves was observed, plant seedlings were transplanted into square pots (length 9.52 cm, height 8.26 cm, and volume 574 cm^3) filled with the Metro-Mix® 360, and uniform plants were selected and moved to the walk-in growth room for the various UV-B and PPFD treatments.

After transplanting, multi-layer cultivating shelves were used with mechanical mini fans (LS1225A-X, AC Infinity, City of Industry, CA, USA) circulating air to achieve uniform temperatures across treatments. Plant canopy temperatures in basil, kale, and mustard plants were maintained at 23.9ºC/21.2ºC, 23.8ºC/21.1ºC, and 23.3ºC/20.1ºC

day/night, respectively. All plants were sub-irrigated as needed with a nutrient solution containing 1.85 $g \cdot L^{-1}$ (277.5 $mg \cdot L^{-1}$ N) 15N-2.2P-12.5K (Peters 15-5-15 Ca-Mg Special, The Scotts Company, Marysville, OH, USA), at electrical conductivity of 2.0 $dS \cdot m^{-1}$ and pH of 6.0.

5.3.2 Supplemental UV-B radiation and PPFD treatments

Exp. I:

Uniform green and purple basil plants were grown under two PPFDs of 160 and 224 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 16-h photoperiod provided by cool white fluorescent lamps (Philips Lighting, Somerset, NJ, USA). Two or five days prior to harvest, UV-B lamps were switched on and basil plants were exposed with one of the five UV-B radiation doses including no supplemental UV-B radiation (control), 1 $h \cdot d^{-1}$ for 2 days (1H2D), 2 $h \cdot d^{-1}$ for 2 days (2H2D), 1 $h \cdot d^{-1}$ for 5 days (1H5D), or 2 $h \cdot d^{-1}$ for 5 days (2H5D) at 16.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (equal to 18.7 $kJ \cdot m^{-2} \cdot h^{-1}$). There were ten treatments (2 PPFD x 5 UV-B) and 12 plants per treatment. Supplemental UV-B radiation treatments were applied at 8:00 am using Philips TL 40W/12 and 20W/12 UV-B broadband lamps (wavelength: 270-400 nm, Svetila.com d.o.o., Domzale, Slovenia, EU). The UV-B light intensity and PPFD in each treatment were measured 15 cm underneath the lamps at 9 spots using MU-200 UV radiation meter (Apogee Instruments, Logan, UT, USA) and PS-100 spectroradiometer (Apogee Instruments, Logan, UT, USA), respectively, before placing the plants. To minimize the disproportionate light distribution within each treatment, all plants were systematically rearranged every three days. All plants were harvested when plant height

reached about 25 cm. The green and purple basil plants were harvested at 19 and 23 days after transplanting (DAT), respectively, equivalent to 40 and 42 days after sowing (DAS), respectively.

Exp. II:

Uniform kale and mustard plants were grown under the same PPFD level of 224 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 16-h photoperiod provided by cool white fluorescent lamps (Philips Lighting, Somerset, NJ, USA). Lower UV-B radiation doses compared to Exp. I were used in this experiment. One to three days prior to harvest, UV-B lamps were switched on and plants were exposed with one of the five UV-B radiation doses including no supplemental UV-B radiation (control), 0.5 $h \cdot d^{-1}$ for 1 day (0.5H1D), 1 $h \cdot d^{-1}$ for 1 day (1H1D), 1 $h \cdot d^{-1}$ for 2 days (1H2D), or 1 $h \cdot d^{-1}$ for 3 days (1H3D) at 16.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$. All the other environmental conditions were the same as Exp. I and plants were harvested when plant height reached about 25 cm. Green and red kale plants were harvested at 17 and 27 DAT (34 and 46 DAS), respectively. Green and red mustard were both harvested at 21 DAT (35 DAS).

5.3.3 Measurements

1. Gas-exchange rate, SPAD index, and chlorophyll fluorescence

A portable gas exchange analyzer (CIRAS-3, PP Systems International, Amesbury, MA, USA) was used to measure the gas exchange rate, including net photosynthetic rate (P_n), transpiration rate (E), and stomatal conductance (G_s) of plant leaves at harvest. A PLC3 leaf cuvette with LED light unit was used. The PPFD, temperature, relative air

humidity, and CO_2 concentration inside the leaf cuvette were set at 800 μmol·m^{-2}·s^{-1}, 25ºC, 50%, and 390 μmol·mol^{-1}, respectively. The third pair of leaves from the top was used for measuring gas exchange rate and measurements were taken when the net photosynthetic rate reached a steady state.

Soil plant analysis development (SPAD) index of plant leaves was recorded at harvest to quantify the relative chlorophyll content of basil leaves using chlorophyll meter SPAD-502 (Konica-Minolta cooperation, Ltd., Osaka, Japan). The third pair of leaves from the top were measured for SPAD. Three measurements were taken for each leaf and the average was recorded for data analysis.

Chlorophyll fluorescence of plant leaves was measured at harvest using a pocket Plant Efficiency Analyzer chlorophyll fluorimeter (PEA, Hansatech Instruments Ltd., Norfolk, UK). The leaves were dark adapted for at least 30 min prior to taking measurements. Minimal fluorescence values in the dark-adapted state (F_0) were obtained by application of a low intensity red LED light source (627 nm), whereas maximal fluorescence values (F_m) were measured after applying a saturating light pulse of 3,500 μmol·m^{-2}·s^{-1}, and maximum quantum use efficiency of photosystem II (PSII) in the dark-adapted state was calculated as $F_v/F_m = (F_m-F_0)/F_m$. The performance index (PI ABS, where "ABS" specifies that the reaction centers' density is expressed per absorption), dissipation of energy per cross section (DI_0/CS), trapped energy flux per cross section (TR_0/CS), and electron transport flux per cross section (ET_0/CS) parameters were calculated using the PEA Plus software (V1.10, Hansatech Instruments Ltd., Norfolk, UK).

2. Growth parameters

Growth parameters such as plant height, width, number of internodes, leaf area, and plant yield including shoot fresh weight (FW) and dry weight (DW) were recorded at harvest. Plant width was calculated as the average of the widest point and its perpendicular width of the basil plant canopy, while leaf length and width in *Brassica* plants were recorded as plant height and width. Leaf area was measured using a leaf area meter (LI-3100, LI-COR, Lincoln, NE, USA). Shoot DW was determined after the shoot tissues were dried at 80ºC in a drying oven (Grieve, Round Lake, IL, USA) for 3 days. Specific leaf area (leaf area per unit leaf dry weight) was calculated as an indicator of leaf thickness.

3. Secondary plant metabolites

Five uniform plants were randomly selected for the measurements of concentrations of anthocyanin, phenolics, and flavonoids, and antioxidant capacity at harvest. Fresh plant leaves were collected in a cooler and immediately stored in a deep freezer (IU1786A, Thermo Fisher Scientific, Marietta, OH, USA) at -80ºC until phytochemical analyses.

Extraction. Approximately 2 g fresh plant leaves were ground in liquid nitrogen and extracted with 15 mL 1% acidified methanol at 4ºC in darkness. After overnight extraction, the mixture was centrifuged (Sorvall RC 6 Plus Centrifuge, Thermo Fisher Scientific, Madison, WI, USA) at 13,200 rpm (26,669 ×*g*) for 15 min, and the supernatant was collected for phytochemical analyses (Xu and Mou, 2016), 2016).

Anthocyanin analysis. The absorbance of extracts was measured at 530 nm using a spectrophotometer (Genesys 10S ultraviolet/Vis, Thermo Fisher Scientific, Madison, WI,

USA), and the anthocyanin concentration was expressed as mg cyanidin-3-glucoside equivalent per 100 g FW of basil leaves using a molar extinction coefficient of 29,600 (Connor et al., 2002). Since the extracts were freshly prepared from leaf tissues maintained at -80ºC and did not undergo extensive processing or significant browning, a pH differential method for anthocyanin content was considered unnecessary (Connor et al., 2002).

Phenolics analysis. The total phenolics concentration of plant leaves was determined using the modified Folin-Ciocalteu reagent method described as the following: 100 µL extraction sample was added to a mixture of 150 µL distilled water and 750 µL 1/10 dilution Folin-Ciocalteu reagent. After 6 min reaction, 600 µL 7.5% Na_2CO_3 was added and the mixture was incubated at 45°C in water bath for 10 min before the absorbance was measured at 725 nm using a microplate reader (ELx800, BioTek, Winooski, VT, USA). Results were expressed as mg of gallic acid equivalent per g FW of basil leaves (Xu and Mou, 2016).

Flavonoids analysis. The total flavonoid concentration of plant leaves was determined as the following: 20 µL extraction sample was added to a mixture of 85 µL distilled water and 5 µL 5% $NaNO_2$. After 6 min reaction, a 10 µL of 10% $AlCl_3 \cdot 6H_2O$ was added to the mixture. After another 5 min reaction, 35 µL of 1M NaOH and 20 µL distilled water were added to the mixture and the absorbance was measured at 520nm using the aforementioned microplate reader (Dou et al., 2018). The results were expressed as mg of (+)-catechin hydrate equivalent per g FW of basil leaves.

Antioxidant capacity analysis. The antioxidant capacity of plant leaves was measured using the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) method (Arnao et al., 2001) described as the following: 150 μL basil leave extract was added to 2.85 mL of $ABTS^+$ solution and incubate at room temperature for 10 min. The absorbance of mixed solution was measured at 734 nm using the aforementioned spectrophotometer. Antioxidant capacity of basil leaves was expressed as mg of Trolox equivalent antioxidant capacity per 100 g FW of basil leaves.

5.3.4 Statistical analysis

Exp. I:

The experiment was arranged in a two factors factorial design. Five plants per treatment were randomly selected for measurements. A two-way ANOVA with two factors, supplemental UV-B radiation and PPFD, and their interaction were analyzed separately for green basil and purple basil plants. After verifying the significance of the two main effects and their interaction, a one-way ANOVA among all treatments was conducted on each variable separately using Student's *t* method. Some data were pooled from two PPFDs because effect of PPFD was not statistically significant. The correlation test between parameters was conducted using Pairwise Correlations method. All statistical analyses were performed using JMP software (Version 13, SAS Institute Inc., Cary, NC, USA). Differences among means were considered significant at $p<0.05$.

Exp. II:

One-way ANOVA among all treatments was conducted on each cultivar using Student's *t* method. The γ between parameters was conducted using Pairwise Correlations method. All statistical analyses were performed using JMP software (Version 13, SAS Institute Inc., Cary, NC, USA). Differences among means were considered significant at $p<0.05$.

5.4 Results

5.4.1 Gas exchange rate, SPAD, and chlorophyll fluorescence

Exp. I:

Gas exchange rates including P_n, E, and G_s in green and purple basil leaves decreased under UV-B treatments, while PPFD showed no effects (Table 15). Treatment 2H5D decreased P_n, E, and G_s in green/purple basil leaves by 68%/70%, 55%/68%, and 65%/76% compared to control, respectively. Similarly, UV-B radiation decreased SPAD readings in green and purple basil leaves by 9-15% and 6-8%, respectively, while PPFD showed no effects on green basil plants but increased the SPAD in purple basil plants (Fig. 14).

All supplemental UV-B radiation treatments decreased F_v/F_m and PI ABS in green basil plants, while in purple basil plants, F_v/F_m showed no differences between control and 1H2D treatment, and PI ABS decreased by the highest UV-B radiation dose, 2H5D treatment (Fig. 15A-B). Similarly, the decreases of TR_0/CS and ET_0/CS were only observed in green basil plants, while purple basil plants showed no differences among all treatments (Fig. 15D-E). In comtrast, DI_0/CS in purple basil plants significantly increased

with higher UV-B radiation dose treatments, 1H5D and 2H5D, while in green basil plants, no treatment effect was observed (Fig. 15C). Chlorophyll fluorescence parameters in green or purple basil plants were not affected by PPFD levels.

Table 15. Net photosynthetic rate (P_n), transpiration rate (E), and stomatal conductance (G_s) of green basil 'Improved Genovese Compact' and purple basil 'Red Rubin' plants under different supplemental UV-B radiation treatments. The five supplemental UV-B radiation treatments included no supplemental UV-B radiation (control), 1 h·d^{-1} for 2 days (1H2D), 2 h·d^{-1} for 2 days (2H2D), 1 h·d^{-1} for 5 days (1H5D), and 2 h·d^{-1} for 5 days (2H5D). Data were pooled from two photosynthetic photon flux density (PPFD) treatments.

Cultivar	Treatment	P_n (μmol·m^{-2}·s^{-1})		E (mmol·m^{-2}·s^{-1})		G_s (mmol·m^{-2}·s^{-1})	
Green basil	Control	13.2	a[z]	2.76	a	130	a
	1H2D	7.8	b	1.74	bc	79	b
	2H2D	8.5	b	1.93	b	93	ab
	1H5D	7.4	b	1.82	b	71	b
	2H5D	4.2	c	1.24	c	46	c
Purple basil	Control	7.4	A	2.73	A	131	A
	1H2D	4.3	B	1.49	B	60	B
	2H2D	3.1	C	1.20	B	42	CD
	1H5D	3.8	BC	1.33	B	49	BC
	2H5D	2.2	D	0.86	C	31	D

[z] Means followed by the same lower/upper case letters are not significantly different, according to Student's *t* mean comparison ($P < 0.05$).

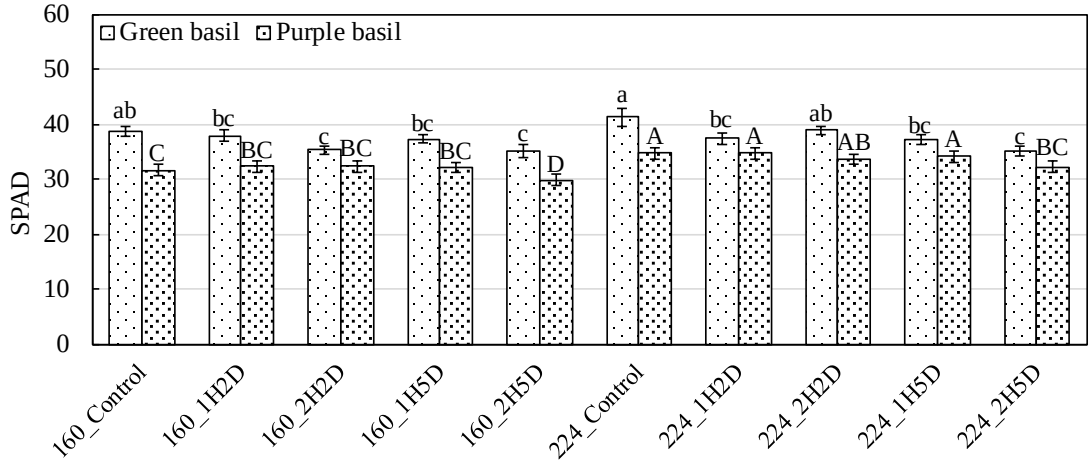

Figure 14. Soil plant analysis development (SPAD) readings of green basil 'Improved Genovese Compact' and purple basil 'Red Rubin' plants at different photosynthetic photon flux density (PPFD) and supplemental UV-B radiation treatments. There were 10 treatments created by the combination of two PPFDs of 160 and 224 μmol·m^{-2}·s^{-1} and five UV-B radiation treatments including no supplemental UV-B radiation (control), 1 h·d^{-1} for 2 days (1H2D), 2 h·d^{-1} for 2 days (2H2D), 1 h·d^{-1} for 5 days (1H5D), and 2 h·d^{-1} for 5 days (2H5D). Means followed by the same lower/upper case letters are not significantly different, according to Student's *t* mean comparison ($P < 0.05$). Bars represent standard errors.

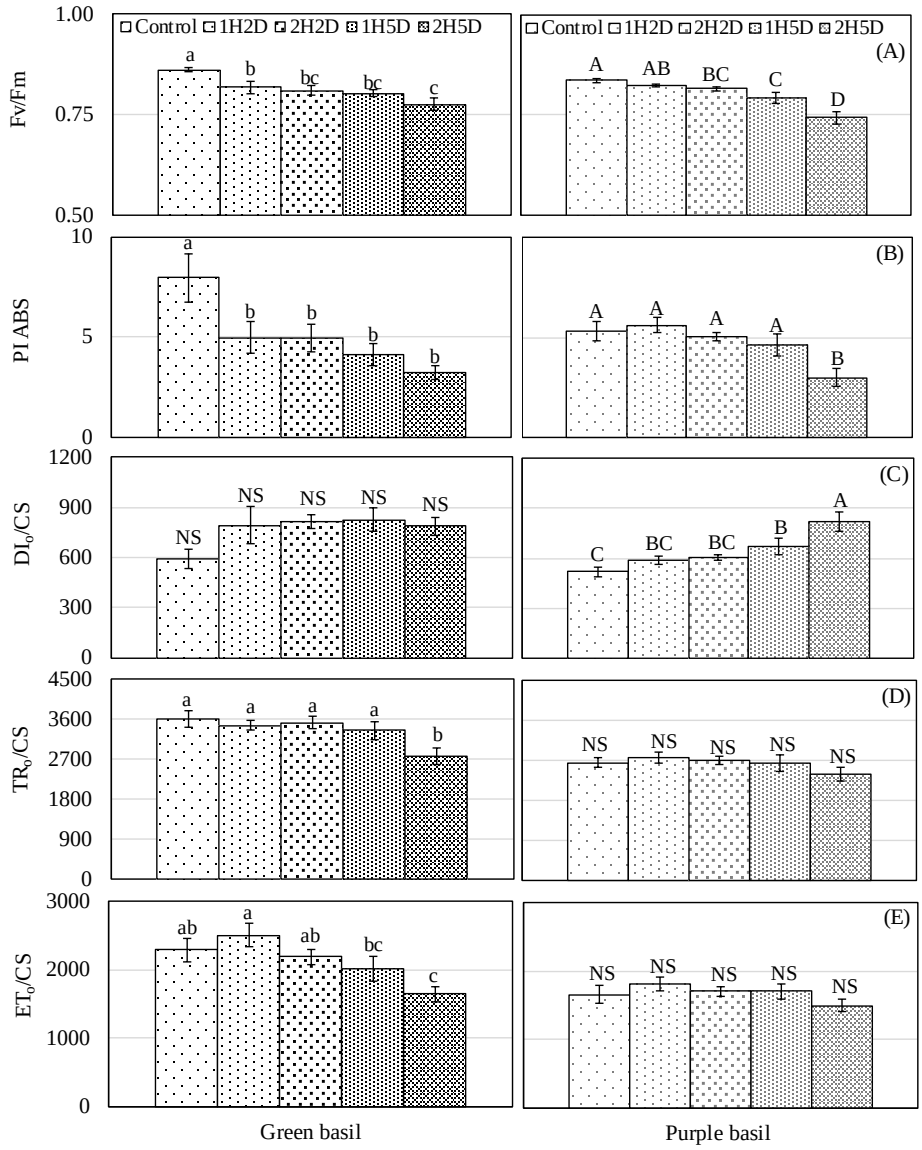

Figure 15. Chlorophyll fluorescence parameters, including maximal photochemical efficiency of Photosystem II (F_v/F_m) (A), performance index (PI ABS, where "ABS" specifies that the reaction centers' density is expressed per absorption) (B), dissipation of energy per cross section (DI_0/CS) (C), trapped energy per cross section (TR_0/CS) (D), and electron transport flux per cross section (ET_0/CS) (E) of green basil 'Improved Genovese Compact' and purple basil 'Red Rubin' plants under different supplemental UV-B radiation treatments including control, 1H2D, 2H2D, 1H5D, 2H5D. Data were pooled from two photosynthetic photon flux density (PPFD) treatments. Means followed by the same lower/upper case letters are not significantly different, according to Student's *t* mean comparison ($P < 0.05$). Bars represent standard errors.

Exp. II:

Supplemental UV-B radiation decreased P_n in all tested plant species except red mustard plants (Fig. 16A). In green kale plants, P_n was the highest under control treatment, followed by 0.5H1D, 1H1D, and 1H2D treatments, and the lowest under 1H3D treatment. In red kale plant, P_n decreased by 1H1D, 1H2D, and 1H3D treatments, but showed no difference between 0.5H1D or control treatments. In green mustard plants, P_n decreased by 1H3D treatment. Supplemental UV-B radiation decreased E regardless of cultivar, with the highest under control treatment, and the lowest under 1H3D treatment (Fig. 16B). Supplemental UV-B radiation did not affect G_s in green or red kale plants. In green mustard plants, G_s was the lowest under 1H1D treatment and showed no differences among other treatments, while in red mustard plants, it was higher under control and 1H2D treatments compared to 0.5H1D, 1H1D, and 1H3D treatments (Fig. 16C). Supplemental UV-B radiation showed no effects on SPAD readings regardless of plant cultivar (data not shown).

Lower UV-B radiation dose treatments, namely 0.5H1D and 1H1D, did not affect F_v/F_m in *Brassica* species compared to control, which higher UV-B radiation dose treatment, namely 1H3D increased F_v/F_m regardless of cultivar (Fig. 17A). Supplemental UV-B radiation did not affect PI ABS in green or red kale plants, while 1H1D and 0.5H1D treatments increased PI ABS in green and mustard plants, respectively (Fig. 17B). In green kale and green mustard plants, DI_0/CS both decreased under 1H1D treatment and increased under 1H3D treatment (Fig. 17C). In red kale and green mustard plants, TR_0/CS decreased under 1H2D, 1H3D and 0.5H1D, 1H1D, 1H3D treatments, respectively (Fig.

17D). Supplemental UV-B radiation did not affect ET_0/CS regardless of cultivar (data not shown).

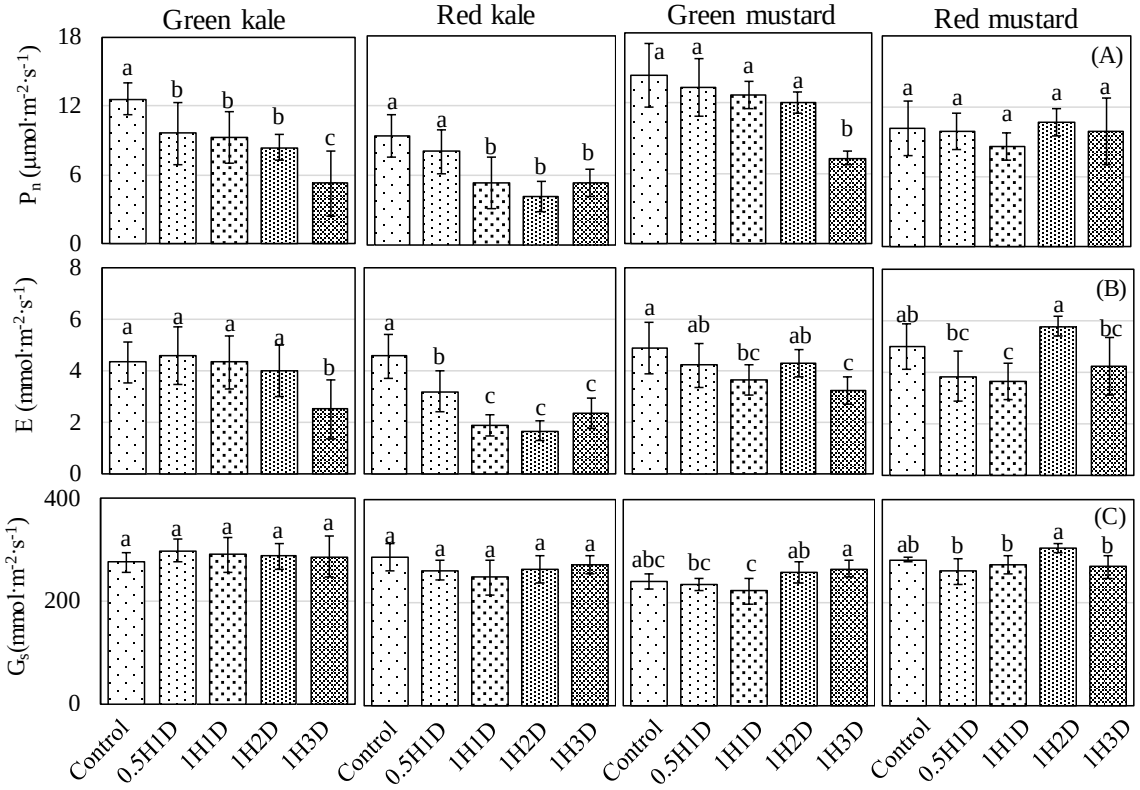

Figure 16. Net photosynthetic rate (P_n) (A), transpiration rate (E) (B), and stomatal conductance (G_s) (C) of green kale 'Siberian', red kale 'Scarlet', green mustard 'Amara', and red mustard 'Red Giant' plants under different supplemental UV-B radiation treatments including no supplemental UV-B radiation (control), 0.5 h·d^{-1} for 1 day (0.5H1D), 1 h·d^{-1} for 1 day (1H1D), 1 h·d^{-1} for 2 days (1H2D), and 1 h·d^{-1} for 3 days (1H3D). Means followed by the same lowercase letter are not significantly different, according to Student's *t* mean comparison ($P < 0.05$). Bars represent standard errors.

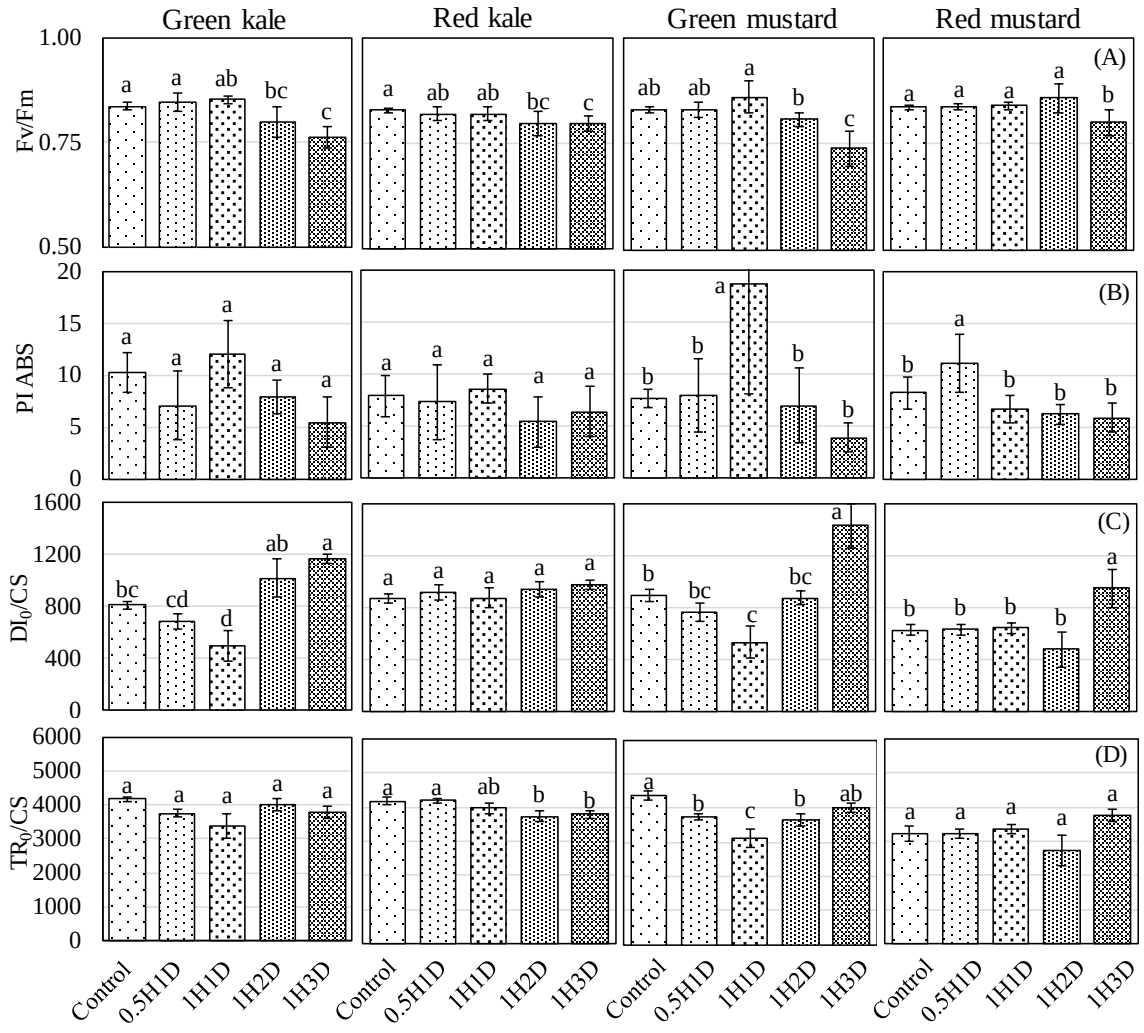

Figure 17. Chlorophyll fluorescence parameters, including maximal photochemical efficiency of Photosystem II (F_v/F_m) (A), performance index (PI ABS, where "ABS" specifies that the reaction centers' density is expressed per absorption) (B), dissipation of energy per cross section (DI_0/CS) (C), and trapped energy per cross section (TR_0/CS) (D) of green kale 'Siberian', red kale 'Scarlet', green mustard 'Amara', and red mustard 'Red Giant' plants under different supplemental UV-B radiation treatments including control, 0.5H1D, 1H1D, 1H2D, 1H3D. Means followed by the same lowercase letter are not significantly different, according to Student's *t* mean comparison ($P < 0.05$). Bars represent standard errors.

5.4.2 Growth parameters and plant yield

Exp. I:

Supplemental UV-B radiation decreased plant height, width, and leaf area in both green and purple basil plants, and the detriment increased with increasing UV-B radiation doses (Table 16). Specifically, under high PPFD (224 $\mu mol \cdot m^{-2} \cdot s^{-1}$), plant height of both green and purple basil plants was the highest under control and 1H2D treatments, followed by 2H2D and 1H5D treatments, and the lowest under 2H5D treatment. Leaf area of green/purple basil plants reduced under all supplemental UV-B radiation treatments, which was 14%/17%, 28%/30%, 28%/34%, and 44%/44% lower under 1H2D, 2H2D, 1H5D, and 2H5D treatments, respectively, compared to control. In contrast, leaf thickness of both cultivars increased with supplemental UV-B radiation, expressed as a decrease in specific leaf area (Table 16). Under higher UV-B exposure doses such as 1H5D and 2H5D treatments, basil plants also showed leaf bronzing, chlorosis, waxy appearance, and premature leaf defoliation (Fig. 18).

Shoot FW and DW of green and purple basil plants generally decreased under supplemental UV-B radiation, and interactions between UV-B radiations and PPFD in shoot FW ($P = 0.01$) and shoot DW ($P = 0.02$) were observed in purple basil plants, while only interactions in shoot DW were observed in green basil plants ($P = 0.03$). Specifically, under low PPFD (160 $\mu mol \cdot m^{-2} \cdot s^{-1}$), 1H2D UV-B treatment showed no effects on shoot FW in green basil plants compared to control, so as the 1H2D and 1H5D treatments in

purple basil plants (Fig. 19A-B). Supplemental UV-B radiation significantly reduced shoot FW of basil plants under high PPFD (224 μmol·m^{-2}·s^{-1}) in both cultivars.

Plant height, leaf area, leaf thickness, and shoot FW and DW in both green and purple basil plants significantly increased at high PPFD (Table 16, Fig. 19A-B). Under control treatment without supplemental UV-B radiation, high PPFD (224 μmol·m^{-2}·s^{-1}) increased plant height, leaf area, leaf thickness, and shoot FW and DW in green and purple basil plants by 16%/12%, 24%/21%, 15%/9%, 44%/34%, and 59%/35%, respectively, compared to low PPFD (160 μmol·m^{-2}·s^{-1}).

Table 16. Two-way ANOVA results for analyzing effects of photosynthetic photon flux density (PPFD), supplemental UV-B radiation, and their interaction (PPFD×UV-B) on plant height, width, leaf area, and specific leaf area of green basil 'Improved Genovese Compact' and purple basil 'Red Rubin' plants.

Cultivar	Treatment	Height (cm)	Width (cm)	Leaf Area (cm^2)	Specific Leaf Area (cm^2·g^{-1})
Green basil	PPFD	***	**	***	***
	UV-B	***	***	***	***
	PPFD×UV-B	NS	NS	NS	**
Purple basil	PPFD	***	NS	***	***
	UV-B	***	***	***	***
	PPFD×UV-B	NS	NS	*	NS

Asterisks (*) indicate significant differences (*P < 0.05; **P < 0.01; ***P <0.001). NS indicates means are not significantly different among treatments (P < 0.05).

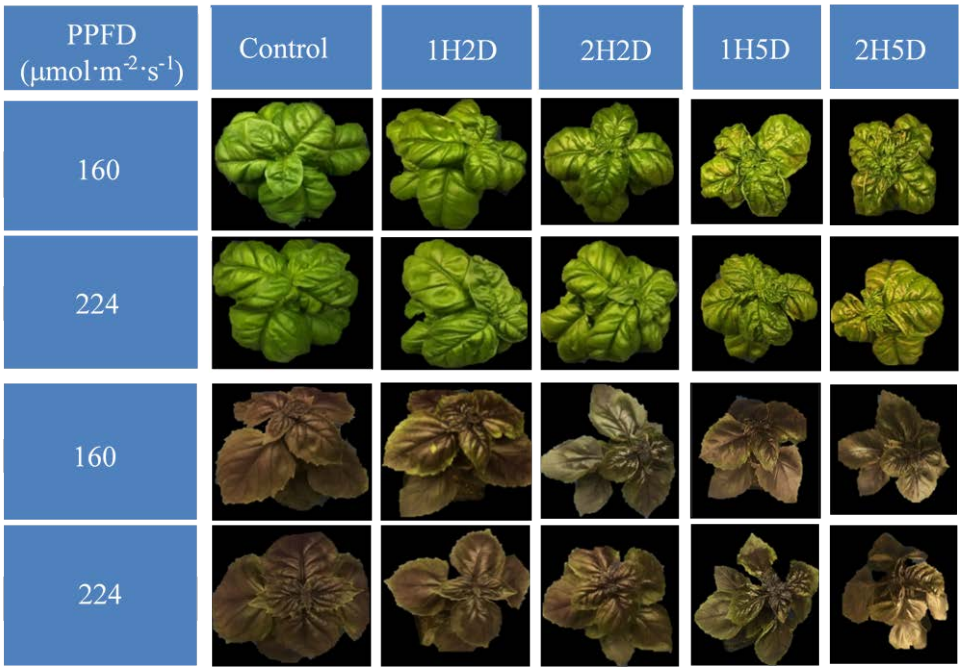

Figure 18. Green basil 'Improved Genovese Compact' and purple basil 'Red Rubin' plants under different photosynthetic photon flux density (PPFD) and supplemental UV-B radiation treatments at harvest. There were 10 treatments created by the combination of two PPFDs of 160 and 224 μmol·m^{-2}·s^{-1} and five UV-B radiation treatments including control, 1H2D, 2H2D, 1H5D, 2H5D.

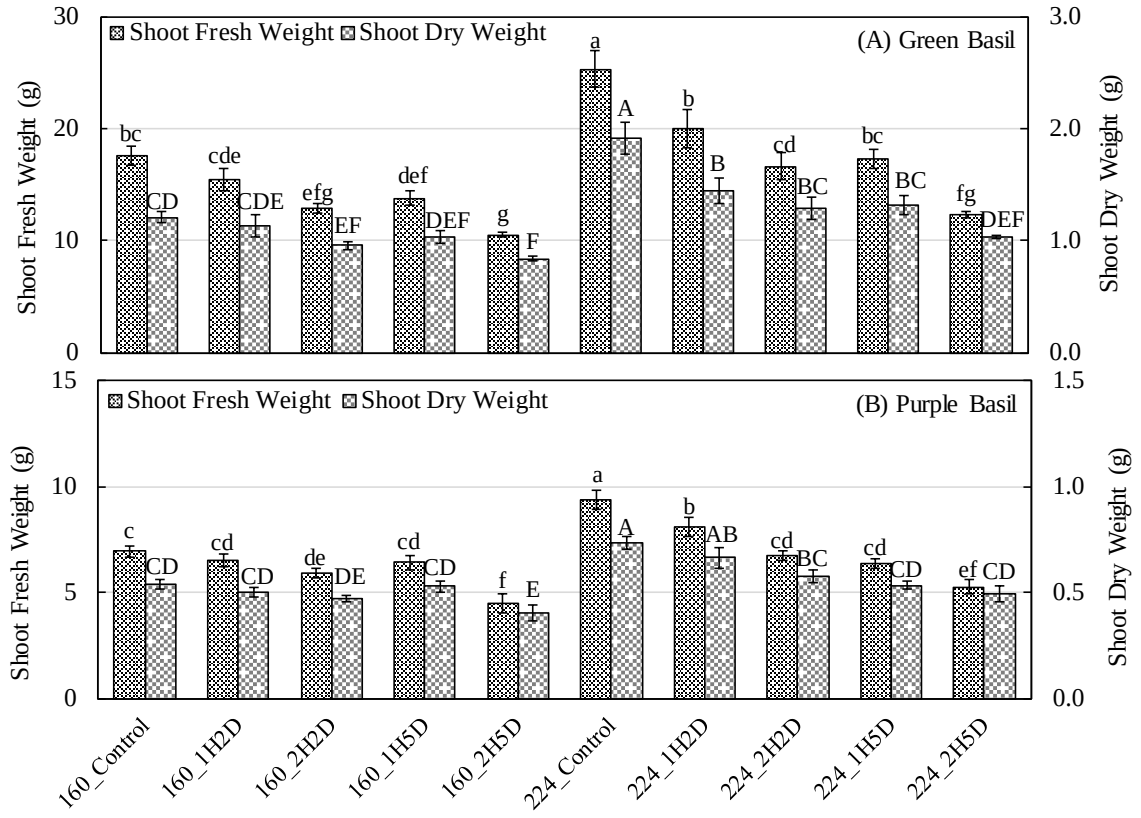

Figure 19. Shoot fresh weight and dry weight of green basil 'Improved Genovese Compact' plants (A) and purple basil 'Red Rubin' plants (B) under different photosynthetic photon flux density (PPFD) and supplemental UV-B radiation treatments. There were 10 treatments created by the combination of two PPFDs of 160 and 224 μmol·m^{-2}·s^{-1} and five UV-B radiation treatments including control, 1H2D, 2H2D, 1H5D, 2H5D. Means followed by the same lower/upper case letter are not significantly different, according to Student's *t* mean comparison ($P < 0.05$). Bars represent standard errors.

Exp. II:

Supplemental UV-B radiation showed no effects on plant height, width, leaf area, leaf thickness, shoot FW, or shoot DW regardless of cultivar, except 0.5H1D treatment increased the shoot DW in green mustard plants (data not shown).

5.4.3 Secondary plant metabolites accumulation and antioxidant capacity

Exp. I:

Supplemental UV-B radiation enhanced phenolic compounds accumulation in basil plants, especially flavonoids concentration in green basil leaves, varying from 80% to 169% compared to control, while anthocyanin and phenolics increased by 9-23% and 28-126%, respectively (Table 17). Anthocyanin and flavonoid concentrations in green basil plants were not influenced by PPFD, while phenolics concentration increased with higher PPFD (Table 17). In purple basil plants, only 2 $h \cdot d^{-1}$ UV-B treatment enriched concentrations of phenolics and flavonoids, while UV-B radiation did not affect anthocyanin concentration (Table 17). Specifically, phenolics and flavonoids concentrations in purple basil plants increased by 29-63% and 37-79% under 2H2D and 2H5D treatments, respectively. High PPFD (224 $\mu mol \cdot m^{-2} \cdot s^{-1}$) increased anthocyanin and phenolics concentrations in purple basil plants but showed no effects on flavonoid concentration (Table 17).

Total amounts of anthocyanin, phenolics, and flavonoids per plant were calculated by multiplying the concentrations of anthocyanin, phenolics, and flavonoids by leaf FW per plant (Table 4). In green basil plants grown under low PPFD (160 $\mu mol \cdot m^{-2} \cdot s^{-1}$), total amount of anthocyanin decreased by 23% under treatment 2H5D, while total amount of phenolics increased by 49% under treatment 2H5D, and total amount of flavonoids increased by 73-79% under treatments 1H2D, 1H5D, and 2H5D (Table 4). In green basil plants grown under high PPFD (224 $\mu mol \cdot m^{-2} \cdot s^{-1}$), total amount of anthocyanin decreased

by 18-39% under treatments 1H2D, 1H5D, and 2H5D, and total amount of phenolics decreased by 15% under treatment 2H5D, while total amount of flavonoids increased by 43-44% under treatments 1H2D and 1H5D (Table 4). In purple basil plants, all supplemental UV-B radiation treatments showed negative or no effects on the total amount of phenolic compounds regardless of PPFD (Table 4).

Antioxidant capacity in green basil plants increased under all supplemental UV-B radiation treatments, while it only increased under 2 $h \cdot d^{-1}$ UV-B treatment in purple basil plants (Fig. 20A). Antioxidant capacity in both green and purple basil plants were positively related to UV-B radiation doses (Fig. 20A). In contrast, purple basil plants after 1 $h \cdot d^{-1}$ UV-B radiation treatments (1H2D and 1H5D) showed no relationship between antioxidant capacity and supplemental radiation dose ($P = 0.1994$), while plants after 2 $h \cdot d^{-1}$ UV-B radiation treatments (2H2D and 2H5D) showed a significant correlation.

Correlations between antioxidant capacity with concentrations of phenolic compounds were analyzed in green and purple basil plants. In green basil plants, concentrations of anthocyanin, phenolics, and flavonoids were all positively related to antioxidant capacity (Fig. 21A). In purple basil plants, concentrations of phenolics and flavonoids were positively related to antioxidant capacity, while anthocyanin concentration showed no relationship ($P = 0.8812$) (Fig. 21B).

Table 17. Anthocyanin concentration, phenolics concentration, and flavonoids concentration in green basil 'Improved Genovese Compact' and purple basil 'Red Rubin' plants at different photosynthetic photon flux density (PPFD) and supplemental UV-B radiation treatments. There were 10 treatments created by the combination of two PPFDs of 160 and 224 μmol·m^{-2}·s^{-1} and five UV-B radiation treatments including control, 1H2D, 2H2D, 1H5D, 2H5D.

Cultivar	Treatment	Anthocyanin Concentration (mg·100g^{-1} FW)		Phenolics Concentration (mg·g^{-1} FW)		Flavonoids Concentration (mg·g^{-1} FW)	
Green Basil	160_ Control	3.19	d[z]	1.10	e	0.45	e
	160_1H2D	3.68	abcd	1.41	de	0.92	cd
	160_2H2D	3.92	a	1.48	d	0.81	d
	160_1H5D	3.49	abcd	1.68	cd	1.00	abcd
	160_2H5D	3.87	ab	2.49	a	1.21	a
	224_ Control	3.29	cd	1.38	de	0.54	e
	224_1H2D	3.39	bcd	2.06	b	0.97	bcd
	224_2H2D	3.78	abc	1.95	bc	0.99	abcd
	224_1H5D	3.35	bcd	2.13	ab	1.15	abc
	224_2H5D	3.89	ab	2.34	ab	1.19	ab
	PPFD	NS		***		NS	
	UV-B	**		***		***	
	PPFD×UV-B	NS		NS		NS	
Purple Basil	160_ Control	10.63	A	2.06	CD	0.94	CD
	160_1H2D	11.02	A	1.63	E	0.82	D
	160_2H2D	10.84	A	2.66	B	1.41	B
	160_1H5D	10.74	A	2.18	C	1.14	C
	160_2H5D	10.75	A	3.35	A	1.68	A
	224_ Control	10.97	A	2.03	CD	1.04	C
	224_1H2D	11.43	A	1.93	CD	1.09	C
	224_2H2D	10.97	A	2.62	B	1.49	B
	224_1H5D	10.85	A	1.85	DE	1.03	C
	224_2H5D	11.07	A	2.85	B	1.42	B
	PPFD	*		*		NS	
	UV-B	NS		***		***	
	PPFD×UV-B	NS		***		**	

[z] Means followed by the same lower/upper case letter are not significantly different, according to Student's *t* mean comparison ($P < 0.05$).

Asterisk (*) indicates significant differences (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). NS indicates means are not significantly different, according to Student's *t* mean comparison ($P < 0.05$).

Table 18. Total amount of anthocyanin, phenolics, and flavonoids per plant in green basil 'Improved Genovese Compact' and purple basil 'Red Rubin' plants at different photosynthetic photon flux density (PPFD) and supplemental UV-B radiation treatments. There were 10 treatments created by the combination of two PPFD of 160 and 224 μmol·m^{-2}·s^{-1} and five UV-B radiation treatments including Control, 1H2D, 2H2D, 1H5D, 2H5D.

Cultivar	Treatment	Total Amount of Anthocyanin (mg·plant^{-1})		Total Amount of Phenolics (mg·plant^{-1})		Total Amount of Flavonoids (CHE mg·plant^{-1})	
Green basil	160_Control	0.47	cde[z]	16.0	d	6.6	d
	160_1H2D	0.47	cde	18.0	d	11.8	b
	160_2H2D	0.42	def	16.0	d	8.8	cd
	160_1H5D	0.40	ef	19.2	cd	11.4	bc
	160_2H5D	0.36	f	23.8	bc	11.6	bc
	224_Control	0.67	a	28.4	ab	10.8	bc
	224_1H2D	0.55	bc	33.2	a	15.4	a
	224_2H2D	0.59	ab	25.6	b	12.8	ab
	224_1H5D	0.52	bcd	31.0	a	15.6	a
	224_2H5D	0.41	ef	24.0	bc	12.2	b
Purple basil	160_Control	0.63	C	12.0	BC	5.6	DE
	160_1H2D	0.58	D	8.6	E	4.2	F
	160_2H2D	0.51	E	12.6	BC	6.0	CDE
	160_1H5D	0.57	D	11.0	CD	5.2	EF
	160_2H5D	0.38	G	11.4	BC	5.6	DE
	224_Control	0.83	A	15.4	A	8.0	A
	224_1H2D	0.72	B	12.2	BC	7.0	ABC
	224_2H2D	0.57	D	13.0	B	7.2	AB
	224_1H5D	0.54	D	9.4	DE	5.4	DE
	224_2H5D	0.47	F	12.2	BC	6.4	BCD

[z] Means followed by the same lower/upper case letters are not significantly different, according to Student's *t* mean comparison ($P < 0.05$).

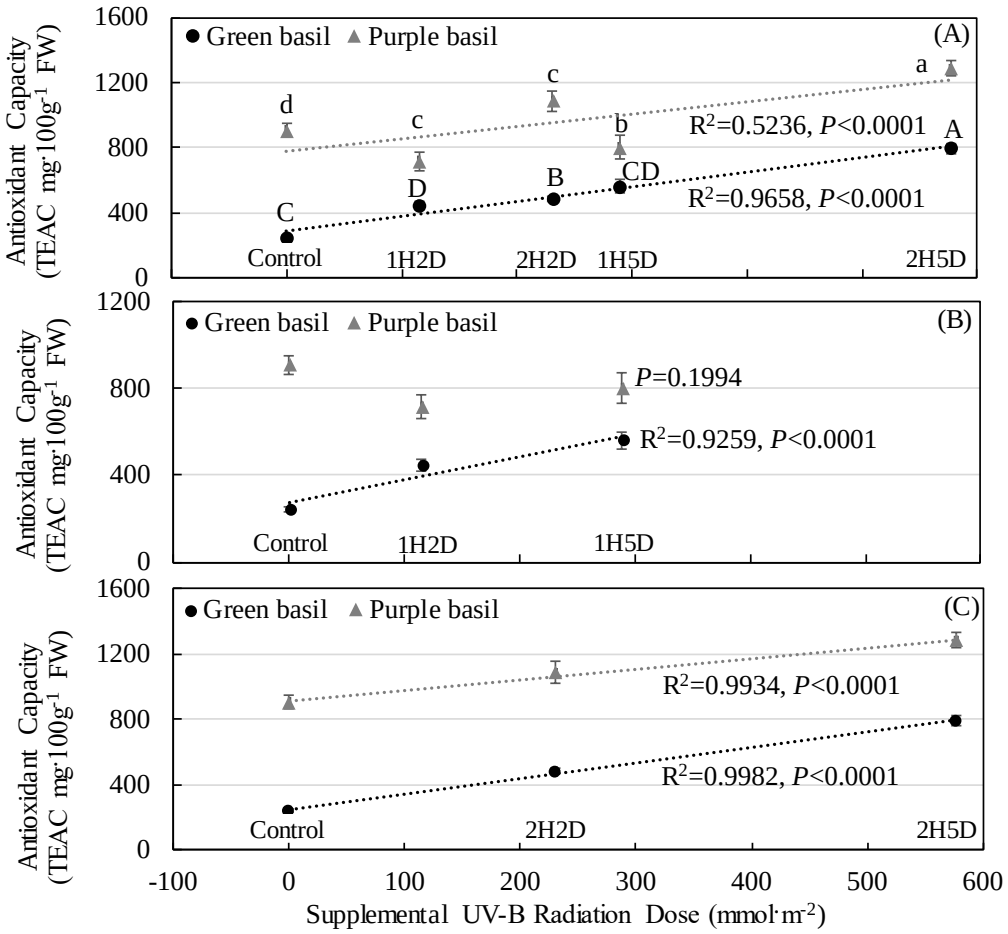

Figure 20. Correlations between antioxidant capacity with five supplemental UV-B radiation treatments including control, 1H2D, 2H2D, 1H5D, 2H5D (A), control and 1 h·d^{-1} UV-B radiation treatments (B), and control and 2 h·d^{-1} UV-B radiation treatments (C) in green basil 'Improved Genovese Compact' and purple basil 'Red Rubin' plants. Antioxidant capacity of basil leaves is expressed as mg of Trolox equivalent antioxidant capacity (TEAC) per 100 g FW basil leaves. Data were pooled from two photosynthetic photon flux density (PPFD) treatments. Means followed by the same lower/upper case letter are not significantly different, according to Student's *t* mean comparison ($P < 0.05$). Bars represent standard errors. Dash lines show regression between antioxidant capacity with supplemental UV-B radiation dose according to Pairwise Correlation method.

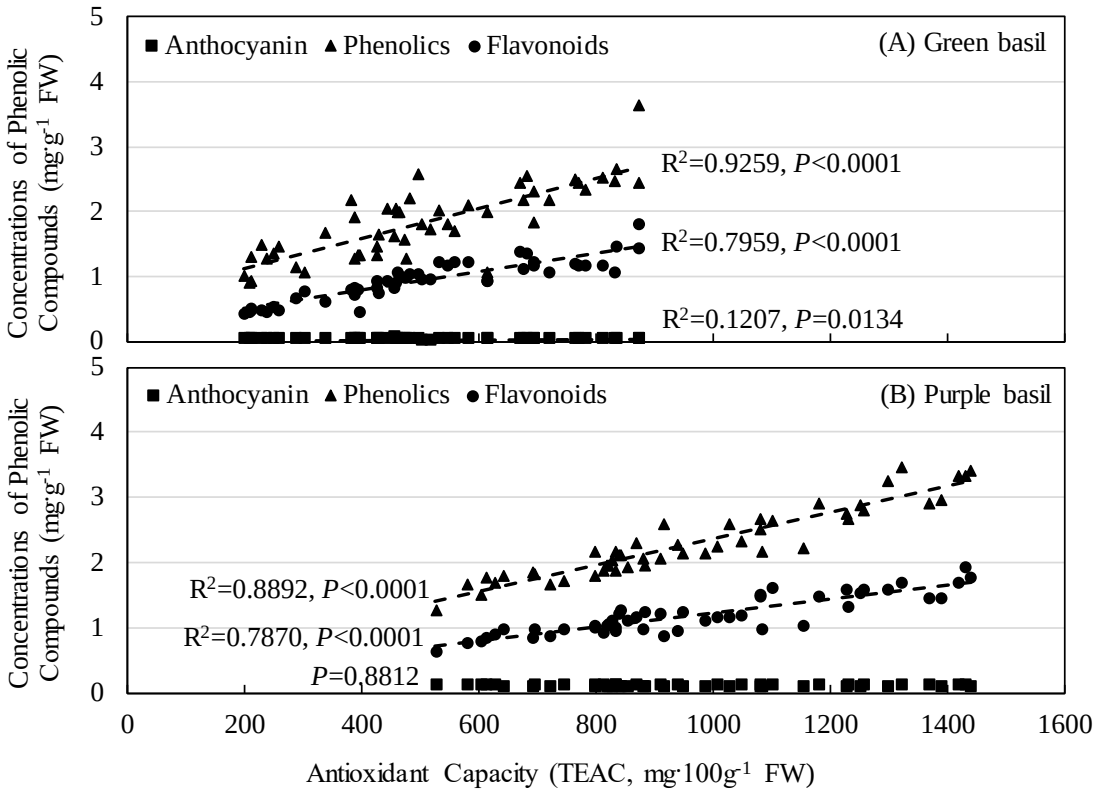

Figure 21. Correlations between antioxidant capacity and concentrations of phenolic compounds including anthocyanin, phenolics, and flavonoids in green basil plants (A), and purple basil plants (B). Antioxidant capacity of basil leaves is expressed as mg of Trolox equivalent antioxidant capacity (TEAC) per 100 g FW basil leaves. Dash lines show regression between concentrations of phenolic compounds with antioxidant capacity according to Pairwise Correlation method.

Exp. II:

Supplemental UV-B radiation showed no effects on concentrations of anthocyanin, phenolics, or flavonoids in green or red kale plants, except 0.5H1D treatment increased anthocyanin concentration in green kale plants (Table 19). In green mustard plants, concentrations of phenolics and flavonoids showed a similar trend, which were the highest under 1H2D and 1H3D treatments, followed by the 0.5H1D and 1H1D treatments, and the

lowest under control, while anthocyanin concentration was not affected by UV-B treatments (Table 19). In contrary, concentrations of phenolics and flavonoids in red mustard plants were not affected by UV-B treatments, while anthocyanin concentration increased under 0.5H1D and 1H1D treatments (Table 19).

Antioxidant capacity in green kale and green mustard plants both increased under 1H2D and 1H3D treatments by 33%-47% and 54%-71% compared to plants grown under control, respectively, and showed no differences among control, 0.5H1D, or 1H1D treatments (Fig. 22). In red kale plants, antioxidant capacity was the highest under 1H3D treatment, followed by 1H2D treatment, and showed no differences among control, 0.5H1D, or 1H1D treatments (Fig. 22). Antioxidant capacity in green kale, red kale, and green mustard plants was all positively correlated with supplemental UV-B radiation doses, while not affected by UV-B treatments in red mustard plants (Fig. 22).

Table 19. One-way ANOVA results for analyzing effects of supplemental UV-B radiation on concentrations of anthocyanin, phenolics, and flavonoids in green kale 'Siberian', red kale 'Scarlet', green mustard 'Amara', and red mustard 'Red Giant' plants.

Treatment	Parameters	Green kale	Red kale	Green mustard	Red mustard
UV-B	Anthocyanin	**	NS	NS	**
	Phenolics	NS	NS	***	NS
	Flavonoids	NS	NS	**	NS

Asterisks (*) indicate significant differences (**$P < 0.01$; ***$P < 0.001$). NS indicates means are not significantly different, according to Student's t mean comparison ($P < 0.05$).

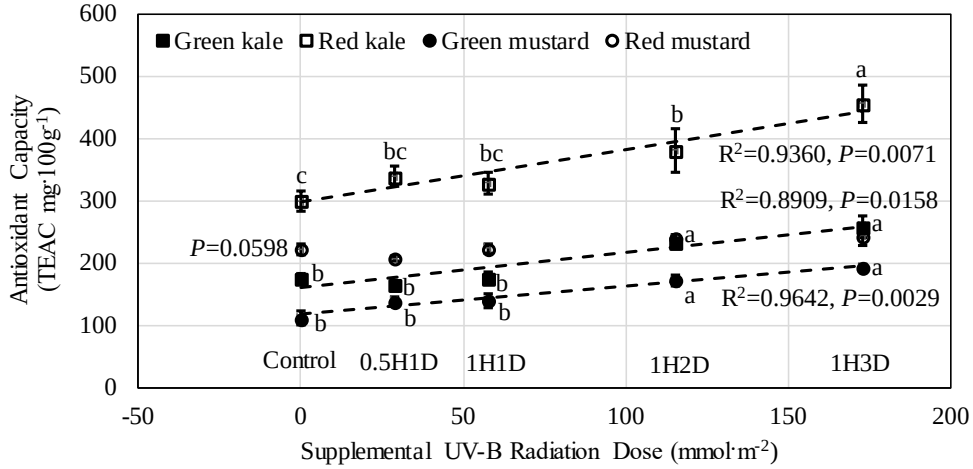

Figure 22. Correlations between antioxidant capacity with five supplemental UV-B radiation treatments including control, 0.5H1D, 1H1D, 1H2D, and 1H3D in green kale 'Siberian', red kale 'Scarlet', green mustard Amara', and red mustard 'Red Giant' plants. Means followed by the same lowercase letter are not significantly different for each cultivar, according to Student's *t* mean comparison ($P < 0.05$). Bars represent standard errors. Dash lines show regression between antioxidant capacity with supplemental UV-B radiation dose according to Pairwise Correlation method.

5.5 Discussion

5.5.1 Impacts of UV-B and PPFD on photosynthesis, SPAD, and chlorophyll fluorescence

Photosynthesis is one of the most sensitive metabolic processes in plants responding to environmental condition changes, such as supplemental UV-B radiation and PPFD. In this study, decreased P_n in basil and *Brassica* leaves under UV-B radiation was mainly caused by direct damage of PSII components, which led to reduced photosynthetic capacity, and subsequently decreased G_s (Sullivan and Teramura, 1990; Lidon et al., 2012; Yadav et al., 2017). Meanwhile, relative chlorophyll content in basil leaves also decreased under UV-

B radiation treatments (Fig. 14), either through degradation or inhibition of enzymes involved in the chlorophyll biosynthetic pathways (Yadav et al., 2017). Decreased gas exchanged rate and SPAD readings in Exp. I compared to unaffected parameters in Exp. II suggested that plant responses to UV-B radiation are species/dose dependent (Table 15, Fig. 14&16).

Unaffected gas exchange rate by PPFD levels in basil leaves may be due to the large variation caused by UV-B radiation at both PPFD treatments: P_n of green basil leaves ranged from 3.7 to 12.6 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at low PPFD (160 $\mu mol \cdot m^{-2} \cdot s^{-1}$), and ranged from 4.8 to 13.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at high PPFD (224 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Compared to depressed photosynthesis and reduced chlorophyll content by UV-B radiation in this study, a meta-analysis of field studies (more than 450 reports from 62 papers) showed that these parameters were not affected by supplemental UV-B radiation (Searles et al., 2001). Differences between our study (controlled environment with artificial lighting) and field studies (sunlight) were probably due to PPFD influencing the response of plants to UV-B treatments. Under controlled environment, due to the high cost of powering artificial lighting, much lower PPFDs are normally used compared to that of sunlight in open field. Accordingly, a depressed photochemical protection system of plants under low PPFD, such as decreased leaf thickness and reduced concentrations of UV-absorbing agents, resulted in severe plant damage by UV-B radiation (Dou et al., 2018).

Chlorophyll fluorescence parameters provide precise and objective data with regard to photochemical efficiency and the processes of non-photochemical de-excitation involved in the conversion of light energy under different conditions (Strasser et al., 2000;

Mosadegh et al., 2018). Less depressed chlorophyll fluorescence activity in purple basil plants (Fig. 17A-B, Fig. 17D-E) clearly indicates its improved capacity to process excess UV-B energy through PSII compared to green basil plants (Rai and Agrawal, 2017). Meanwhile, uninfluenced DI_0/CS under UV-B treatments in green basil plants suggests its inability to dissipate the absorbed UV-B radiation energy as harmless heat, even with the smallest UV-B radiation dose, 16.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 1 $h \cdot d^{-1}$ for 2 days, while purple basil plants after high UV-B radiation doses (1H5D and 2H5D treatments) coped with excess energy by increasing the rate of heat dissipation. Similarly, Mosadegh et al. (2018) also reported that the DI_0/CS of green basil plants after 2-weeks supplemental UV-B radiation at 68 and 102 $kJ \cdot m^{-2} \cdot d^{-1}$ showed no difference from control, indicating a failure to dissipate UV-B energy as heat. Under lower UV-B radiation doses in Exp. II, the firstly decreased DI_0/CS after 1-day UV-B treatment then increased DI_0/CS after 2 or 3-days UV-B treatment in green kale and mustard plants indicated plants could adapt to UV-B radiation by improving their heat dissipation within 2 or 3 days (Fig. 17C).

Differences in chlorophyll fluorescence parameters between green and purple/red leaf plants may be due to higher concentrations of UV-protective antioxidants in purple/red leaves (Table 17, Fig. 22), which are known to provide plants with stronger protection from excess UV-B radiation (Takahashi and Badger, 2011). Noticeably, green basil plants after different UV-B radiation treatments at similar doses (2H2D and 1H5D treatments) showed no differences in F_v/F_m, PI ABS, TR_0/CS, or ET_0/CS, indicating that photochemistry responses of green basil plants to UV-B radiation are more dose dependent

instead of radiation patterns (different combinations of radiation period per day and radiation days), which is also thought to be true in purple basil plants.

5.5.2 Impacts of UV-B and PPFD on plant growth and development

Plant leaf expansion is invariably inhibited by UV-B radiation and other leaf morphogenesis changes such as reduced leaf area, increased leaf thickness, and accumulation of leaf surface waxes are also observed across a number of plant species (Cen and Bornman, 1993; Jansen and Bornman, 2012; Wargent and Jordan, 2013). Similar to studies on other species, both green and purple basil plants in this study displayed a reduced leaf area with increased leaf thickness by supplemental UV-B radiation (Table 16), which may provide plants improved tolerance to other stress factors, such as mechanical handling during postharvest (Wargent et al., 2009). Similarly, waxy appearance of leaf surface in both green and purple basil plants indicated increased epicuticular wax deposit in basil leaves (Kakani et al., 2003), which can also provide basil leaves protection from excess UV-B radiation and other adverse environmental conditions.

Internode length is a very sensitive growth parameter that responds to UV-B radiation (Zhao et al., 2003). Kaiserli (2018) showed that most cell-wall elongation genes induced by BRI1-EMS-SUPPRESSOR 1 are negatively regulated by UV-B radiation. Meanwhile, the biosynthesis and signaling of plant growth hormone auxin, a key regulator of stem elongation, was also suppressed in arabidopsis and coriander (*Coriandrum sativum*) after UV-B radiation, thereby reducing plant stem elongation and promoting a compact

phenotype (Fraser et al., 2017). Similarly, in this study, plant height was shorter under supplemental UV-B radiation compared to plants under control treatment with similar internode number (data not presented).

Decreased gas exchange rate, reduced leaf expansion, and inhibition of stem elongation of basil plants under supplemental UV-B radiation resulted in smaller plant size (Fig. 18) and decreased shoot FW and DW (Fig. 19A-B). The greater yield reduction by UV-B radiation under high PPFD (224 $\mu mol \cdot m^{-2} \cdot s^{-1}$) may be due to its taller plants, which shortened the distance between basil plants and UV-B light tube compared to low PPFD (160 $\mu mol \cdot m^{-2} \cdot s^{-1}$), resulting in increased UV-B radiation intensity sustained by basil plants. Plant responses to supplemental UV-B radiation including inhibited leaf expansion and stem elongation, increased leaf thickness, accumulation of leaf surface wax, and leaf defoliation serve together as a protective mechanism to protect basil plants from receiving excess UV-B radiation.

In Exp. II, there was a discrepancy between the effects of UV-B radiation on plant photosynthesis and growth. Although plant gas exchange rate decreased under supplemental UV-B radiation, plant growth parameters (i.e., leaf length, width, leaf area, leaf thickness) or biomass accumulation (i.e., shoot FW and DW) were not influenced, suggesting that photosynthesis was the most sensitive response and was primarily affected in plants after UV-B radiation. Combining results from Exp. I and Exp. II, supplemental UV-B radiation had negative or no effects on plant growth or yield depending on UV-B radiation doses, specifically, plant growth and yield won't be decreased by preharvest, short term, and relatively low UV-B radiation dose treatment.

5.5.3 Impacts of UV-B and PPFD on phenolics accumulation and antioxidant capacity

Across a range of plant species, phenolic compounds, especially flavonoids, act as efficient UV-screening agents to reduce the excess UV light received by photosynthetic tissues to protect plant from possible harm (Takahashi and Badger, 2011; Logan et al., 2015). Enhanced accumulation of phenolic compounds by UV-B treatments has been supported by a large body of experimental evidences (Agati and Tattini, 2010; Hatier et al., 2013), which was also confirmed in this study (Tables 17, 19). Upon supplemental UV-B radiation, gene expression of phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS), two key molecular markers for phenolic compounds biosynthesis increased significantly (Fraser et al., 2017; Rodriguez-Calzada et al., 2019). Ghasemzadeh et al. (2016) also reported that a 13 $kJ \cdot m^{-2} \cdot h^{-1}$ post-harvest UV-B radiation for 4-10 h increased the total phenolic and flavonoid content by 16% and 85% in green basil plants, respectively, and no anthocyanin content was measured. Enhancement of flavonoids and phenolics by UV-B radiation was greater compared to the increase of anthocyanin in basil plants (Table 17). Consistently, improved antioxidant capacity by UV-B radiation was mainly attributed to concentrations of phenolics and flavonoids in both green and purple basil plants, and marginally to the anthocyanin concentration in green basil plants, while not related to the anthocyanin concentration in purple basil plants (Fig. 23A-B). Csepregi et al. (2017) also reported such differential regulation of different phenolic compounds by UV-B radiation, which was probably due to higher ROS-scavenging capacity of phenolics and flavonoids than anthocyanins.

Responses of secondary metabolites accumulation to supplemental UV-B radiation in *Brassica* plants are species specific. Concentrations of phenolics and flavonoids in green mustard plants increased under UV-B radiation, whereas antioxidant capacity in green kale, red kale, and green mustard plants all increased under UV-B radiation (Fig. 22), indicating the synthesis of other antioxidants in kale plants were stimulated by UV-B radiation instead of phenolics and flavonoids. For example, Nasibi and M-Kalantari (2005) reported that the thiobarbituric acid reactive substances (TBARS, a reliable indicator of free radical formation in plant tissues), ascorbic acid, dehydroascorbic acid, and total ascorbate in kale plants increased significantly under supplemental pre-harvest UV-B radiation for 21 days, in addition to flavonoids. Similarly, the total phenolics, flavonoids, and ascorbic acid in broccoli plants increased by 14%-75%, 4%-13%, and 67%-115%, respectively, after different pre-harvest UV-B radiation doses for 76 days (Topcu et al., 2015). Both studies reported enrichment of other phytochemicals by UV-B radiation in addition to phenolics and flavonoids, but both studies had significantly longer UV-B radiation periods than the present study, and neither of them characterized the effects of UV-B radiation on biomass accumulation. We postulate that low radiation dose and/or short radiation period is the reason of unaffected concentrations of phenolics and flavonoids in green or kale plants, and the clarification of enriched antioxidant(s) in kale plants by UV-B radiation need further investigation.

Relatively high concentrations of phenolic compounds in purple/red leaf plants acted as potent UV-screening agents as well as free-radical scavengers to protect purple/red leaf plants from excess UV-B light and led to less biochemical changes under UV-B radiation

compared to green leaf plants (Tables 17, 19). Under high PPFD (224 μmol·m^{-2}·s^{-1}) without UV-B treatment, concentrations of anthocyanin, phenolics, and flavonoids in purple basil leaves were 3.33, 1.47, and 1.93 times of those in green basil leaves, respectively (Table 17), while its antioxidant capacity was 3.72 times that in green basil leaves (Fig. 21A). Similarly, antioxidant capacity in red kale and red mustard plants were 1.70 and 1.98 times of those in green kale and green mustard plants, respectively. Tattini et al. (2014) also reported that purple basil 'Red Rubin' showed lower metabolic cost of photoprotective mechanisms and higher biomass increase than green basil 'Tigullio' when being moved from 30% to 100% sunlight condition.

In our previous study, concentrations of phenolics and flavonoids in green basil leaves were positively related to PPFD (Dou et al., 2018), and a similar trend was observed in phenolics concentration in this study. Under low PPFD (160 μmol·m^{-2}·s^{-1}), enhancement of phenolic compounds in both green and purple basil plants caused by UV-B radiation was greater compared to plants grown under high PPFD (224 μmol·m^{-2}·s^{-1}), indicating basil plants are more sensitive to UV-B radiation under low PPFD. In a similar way, Behn et al., (2010) reported that under low PPFD (550 μmol·m^{-2}·s^{-1}), essential oil quality in peppermint plants was improved in terms of enhanced menthone to menthol conversion by UV-B exposure, while not affected by UV-B radiation under high PPFD (1,150 μmol·m^{-2}·s^{-1}). As aforementioned, this may be due to a depressed protection system of plants grown under low PPFD, such as decreased leaf thickness and reduced concentrations of UV-absorbing agents (Dou et al., 2018). Meanwhile, concentrations of

flavonoid in green basil plants grown under low PPFD with UV-B radiation was significantly higher compared to those of plants grown under high PPFD without UV-B radiation, suggesting that UV-B radiation compensated for the reduced accumulation of phytonutrients in green basil plants grown under low PPFD.

5.5.4 Impacts of UV-B radiation doses and radiation patterns on phenolics accumulation and antioxidant capacity in basil plants

Plant responses to supplemental UV-B radiation are cultivar specific. With the radiation doses and patterns used in this study, green basil plants were more dose dependent, while purple basil plants were more radiation pattern dependent. The antioxidant capacity in green basil plants was significantly correlated with UV-B radiation doses for both 1 $h \cdot d^{-1}$ and 2 $h \cdot d^{-1}$ UV-B radiation treatments (Fig. 21B-C), indicating total UV-B radiation dose was the determining factor in regulating plant biochemical responses to UV-B radiation. Mosadegh et al. (2018) also reported that with the same UV-B radiation dose of 102 $kJ \cdot m^{-2}$, phenolics concentration of green basil 'Genovese' was the same level at 24 h, 48 h, and 72 h after two UV-B radiation patterns, continuous 1-d UV-B radiation and discontinuous 6-d UV-B radiation treatments. However, at 72 h after UV-B radiation doses of 8.5, 34, and 68 $kJ \cdot m^{-2}$, phenolics concentration of 'Genovese' basil plants treated with continuous 1-d UV-B radiation increased by 239%, 193%, and 139% compared to those of plants treated with discontinuous 6-d UV-B radiation, respectively. Thus, the effects of radiation patterns on green basil plants may vary according to different radiation doses. Different from green basil plants, the antioxidant capacity of purple basil plants

showed no relationship with 1 $h \cdot d^{-1}$ UV-B radiation treatments while being positively related to 2 $h \cdot d^{-1}$ UV-B radiation treatments (Fig. 21B-C), indicating radiation patterns had more effects on purple basil plants' responses to UV-B radiation instead of the total UV-B radiation dose. With similar UV-B radiation dose (1H5D and 2H2D treatments), after 1 $h \cdot d^{-1}$ UV-B radiation treatments, the recovery time (23 h) until next day treatment allowed purple basil plants' signaling and metabolic adaptation to (at least partially) reset to prestress levels, without increasing phenolic compounds accumulation, while after 2 $h \cdot d^{-1}$ UV-B radiation (recovery time of 22 h until next treatment), purple basil plants failed to recover from UV-B radiation stress and resulted in an overall increase of phenolic compounds to cope with excess UV-B energy.

5.5.5 Implications of study findings

Plant responses to UV-B radiation are different in studies conducted in open field with sunlight and controlled environment with artificial lighting, due to different PPFDs (Searles et al., 2001; Li et al., 2010; Henry-Kirk et al., 2018). That is, under controlled environment with artificial lighting, plants are grown under much lower PPFDs, compared to sunlight. When treated with the same dose of UV-B radiation as that of sunlight, plants are more sensitive to supplemental UV-B radiation, and the negative effects were aggravated (Behn et al., 2010; Wargent et al., 2011). Therefore, for controlled environment crop production with low PPFDs, a lower UV-B radiation dose should be applied to reduce its negative effects in plant photosynthesis, growth, or yield.

Plant responses to supplemental UV-B radiation lead to plant cross-protection against other environmental stresses, through both morphological and biochemical mechanisms (Yin and Ulm, 2017). For example, UVR8 was recently shown to be involved in regulating thermomorphogenesis, shade-avoidance response, and plant immunity, underlining the importance of signaling crosstalk among UV light, hormone, and defense pathways (Teklemariam and Blake, 2003; Schultze and Bilger, 2019). As a result, in addition to plant nutritional quality improvement, supplemental UV-B radiation can also be applied to horticultural crops to improve plant tolerance to other adverse environmental conditions. However, the interactions between supplemental UV-B radiation and other key environmental conditions still need to be studied.

Furthermore, we see differential responses in green and purple basil plants to supplemental UV-B radiation. The accumulation of phenolic compounds in green basil plants mainly depended on the total UV-B radiation dose, while in purple basil plants, radiation patterns had more effects. Therefore, to better understand plant responses to supplemental UV-B radiation, more plant species and cultivars and radiation doses and patterns should be investigated.

5.6 Conclusion

Results of this study suggest that plant responses to UV-B radiation are cultivar, radiation dose, and radiation pattern dependent. Specifically, all supplemental UV-B radiation doses efficiently improved concentrations of secondary metabolites and antioxidant capacity in green basil, green kale, red kale, and green mustard plants while

higher UV-B radiation doses significantly reduced plant size and yield in basil plants and lower doses showed no effects on plant growth or biomass accumulation in *Brassica* plants. Meanwhile, effects of UV-B radiation on basil plants interacted with PPFD, which is, low PPFD increased plant sensitivity to UV-B radiation and UV-B radiation compensated for the reduced accumulation of flavonoids in green basil plants grown under low PPFD. In conclusion, a pre-harvest UV-B radiation (16.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$) of 1 $h \cdot d^{-1}$ for 2-3 days under a PPFD of 224 $\mu mol \cdot m^{-2} \cdot s^{-1}$ could enrich plant secondary metabolites accumulation without reducing biomass accumulation, which was recommended for green basil and *Brassica* plants production under controlled environment.

CHAPTER VI

SUBSTITUTING PHOTOSYNTHETICALLY ACTIVE RADIATION LIGHT WITH FAR-RED LIGHT INCREASED BIOMASS AND SECONDARY METABOLITES ACCUMULATION IN BASIL PLANTS

6.1 Synopsis

Although far-red light is poorly absorbed compared to photosynthetically active radiation (PAR) light, recent research indicated that supplemental far-red light to PAR light or substituting far-red light for PAR light increases plant yield of lettuce (*Lactuca sativa*), kale (*Brassica oleracea*), and several ornamental seedlings because far-red light induces plant expansion growth and a better-balanced excitation of the two photosystems. Therefore, the effects of substituting PAR light with far-red light was investigated in the present study in green basil (*Ocimum basilicum* 'Improved Genovese Compact') plants. There were five treatments without far-red light substitution, including $R_{53}B_{47}$, $R_{80}B_{20}$, $R_{91}B_{9}$, $R_{42}G_{43}B_{12}FR_{3}$, and $R_{33}G_{40}B_{24}FR_{3}$ (subscripted numbers indicating percentage; R, red light; G, green light; B, blue light; FR, far red light). Five far-red light substitution treatments were created by adding R/FR light tubes to each aforementioned light treatment, including $R_{47}B_{40}FR_{13}$, $R_{66}B_{21}FR_{13}$, $R_{80}B_{7}FR_{13}$, $R_{36}G_{37}B_{10}FR_{17}$, and $R_{27}G_{33}B_{20}FR_{20}$, a total of ten treatments. The experiment was conducted in a growth room with the same total photon flux density (TPFD) of 230 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 16-h photoperiod. Plants were sub-irrigated as needed using a nutrient solution with electrical conductivity of 2.0 $dS \cdot m^{-1}$ and pH of 6.0. Results indicated that substituting partial PAR light with far-red light increased plant height and width in basil plants by 49%-65% and 10%-17%, respectively, and increased shoot fresh weight and dry weight by 6%-23% and 4%-28%, respectively. However, far-

red light substitution did not affect leaf photosynthesis or leaf area, but decreased chlorophyll content. Concentrations of anthocyanin, phenolics, and flavonoids of basil leaves increased or tended to increase under far-red light treatments, while antioxidant capacity increased by 17%-44% under far-red light treatments except treatment $R_{36}G_{37}B_{10}FR_{17}$.

6.2 Introduction

Photosynthetically active radiation (PAR, 400-700 nm), including blue (400-499 nm), green (500-599 nm), and red (600-699 nm) light wavelengths, is crucial for plant growth with respect to providing light energy for photosynthesis and as a signal to regulate plant adaptive responses to environment (Dou et al., 2017; Snowden et al., 2016; Son et al., 2017). Radiation outside the PAR range, such as ultraviolet (UV, 280-399 nm) and far-red (700-780 nm) light, regulates numerous signaling pathways in plants (Ballaré, 2014; Casal, 2013; Wargent and Jordan, 2013). For example, in our previous study in Chapter V, pre-harvest UV-B radiation induced antioxidants synthesis and other protective mechanisms in basil and *Brassica* plant species. Far-red light is poorly absorbed compared to PAR light, and most is transmitted through or reflected by leaves. For instance, the red: far-red (R:FR) ratio of sunlight is around 1.0 to 1.3 at midday and varies little with different weathers or seasons, but it can be as low as less than 0.1 underneath a plant canopy (for example, it was 0.033 under a sugar-beet canopy) (Holmes and Smith, 1975; Pedmale et al., 2016). Recent studies reported that far-red light can also affect plant productivity and nutritional quality via regulation of plant photosynthesis and photomorphogenesis (Demotes-Mainard et al., 2016; Meng et al., 2019; Yang et al., 2013; Zhen and Van Iersel, 2017).

Far-red light is best known for its role in shade avoidance responses, which is mediated by phytochrome photoreceptors. There are two reversible forms of phytochromes, the biologically

inactive form P_r (for red light absorbing, peaks at 660 nm) and active form P_{fr} (for far-red light absorbing, peaks at 730 nm) (Quail, 2002). The phytochrome photoequilibrium (PPE, also called photostationary state of phytochrome, PSS), which estimates the proportion of P_{fr} in total phytochromes, dynamically changes with the composition of light spectrum, and is strongly correlated with R:FR ratio (Mccree, 1972; Sager et al., 1988). A low R:FR ratio is indicative of shade environment that triggers plant shade avoidance responses, such as elongation growth, upward leaf orientation, and reduced branching (Meng and Runkle, 2017). These growth and developmental responses can enable plants to outgrow shade and capture more photosynthetic radiation, subsequently increase plant yield. For example, Meng and Runkle (2017) reported that adding far-red to combined red and blue (R&B) light increased leaf size and fresh weight (FW) in lettuce (*Lactuca sativa*) and basil plants but decreased the relative chlorophyll content in lettuce plants. However, some researchers hypothesized that the substitution of PAR light wavelengths with far-red light may decrease whole-plant photosynthetic efficiency due to a decreased photosynthetic photon flux density (PPFD), which decreases plant yield (Demotes-Mainard et al., 2016). Park and Runkle (2017) reported that adding far-red light to combined R&B light increased the plant height, leaf area, and shoot dry weight (DW) in geranium (*Pelargonium* × *hortorum*) and snapdragon (*Antirrhinum majus*) seedlings significantly, while substituting far-red light for red light increased leaf area but showed no differences on shoot DW. It was also reported that low R:FR ratio decreased production of phytochemicals in plants, such as jasmonic acid and anthocyanins (Ballaré, 2014; Holopainen et al., 2018; Kadomura-Ishikawa et al., 2013). Therefore, how far-red interacts with PAR light wavelengths affecting plant production is still unclear.

The low quantum yield of photosynthesis under far-red light is caused by unbalanced excitation of two photosystems, photosystem I (PSI) and photosystem II (PSII), which is preferentially excited by far-red and PAR light, respectively (Allen, 2003; Emerson and Rabinowitch, 1960; Myers, 1971). It is postulated that the excitations of two photosystems are unbalanced in plants grown under controlled environment with artificial lighting, where far-red light is absent. Zhen and Van Iersel (2017) reported that the quantum yield of PSII and net photosynthetic rate in 'Green Towers' lettuce plants increased immediately by adding far-red light to combined R&B light ($B_{23}G_1R_{76}$) and white light ($B_{12}G_{43}R_{41}FR_4$), owning to a better balanced excitation of PSI and PSII. They suggested that far-red light and PAR light can have synergistic effects on photochemistry and photosynthesis, and far-red light is needed for efficient photochemistry, especially under light with wavelengths that over-excite PSII (Zhen and Van Iersel, 2017).

There is potential of using far-red light to obtain desirable morphological traits and improve plant photosynthesis, and subsequently increase plant yield. Therefore, the objective of this study was to investigate the effects of substituting far-red light for PAR wavelengths on photosynthesis, morphology, plant yield, and nutritional quality in basil plants.

6.3 Materials and Methods

6.3.1 Plant materials and growing conditions

An experiment was conducted in a walk-in growth room in Texas AgriLife Research and Extension Center at El Paso, TX using green basil 'Improved Genovese Compact' (Johnny's Selected Seeds, Winslow, ME, USA). One seed per cell was sown in 72 square cell trays (cell size: 3.86 cm L × 5.72 cm H, with a volume of 59 cm^3) with Metro-Mix 360 (peat moss 41%, vermiculite 34%, pine bark 25%, Sun Gro® Horticulture, Bellevue, WA, USA). All trays were put

under mist in a greenhouse for germination. Seedlings were moved out from mist after germination and grown in a greenhouse for two weeks. Seedlings were then transplanted to 4" square pots (length 9.52 cm, height 8.26 cm; volume 574 cm^3) with Metro-Mix 360 when roots were visible on the outside of the plug root ball, and uniform plants were selected and moved to the walk-in growth room for different treatments.

6.3.2 Far-red light treatments

There were ten different light quality treatments comprised of blue, green, red, and far-red light (Table 20). The first group of five treatments had no far-red light substitution, and consisted of combinations of R&B light emitting diode (LED) treatments, namely $R_{53}B_{47}$ (Model GEHL48HPPV, Hort Americas, Bedford, TX, USA, where the percentage of red and blue light was 53% and 47%, respectively), $R_{80}B_{20}$ (Model GEHL48HPPB), and $R_{91}B_9$ (Model GEHL48HPPR); one white LED treatment $R_{42}G_{43}B_{12}FR_3$ (Model GEHL48HWTB); and one white fluorescent lamp treatment $R_{33}G_{40}B_{24}FR_3$ (Philips Lighting, Somerset, NJ, USA). The second group of five treatments consisted of substituting far-red light for partial PAR light via adding R/FR light tubes (Just Power Integrated Technology Inc., Taiwan) to each light quality treatment used in the first group. The treatments included $R_{47}B_{40}FR_{13}$, $R_{66}B_{21}FR_{13}$, $R_{80}B_7FR_{13}$, $R_{36}G_{37}B_{10}FR_{17}$, and $R_{27}G_{33}B_{20}FR_{20}$. The total photon flux density (TPFD, 400-780 nm) of each treatment were adjusted to the same level of 230 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 16-h photoperiod. There were 12 plants per treatment. To minimize light distribution being disproportionate within each treatment, all plants were systematically rearranged every three days. The photon flux density in each treatment was measured at 15 cm underneath the light lamps at 9 spots using PS-100 spectroradiometer (Apogee Instruments, Logan, UT, USA). Estimated PPE and yield photon flux

density (YPFD, product of photo flux density and relative quantum efficiency) were calculated by the SpectraWiz software (v5.3, StellarNet Inc., Tampa, FL, USA).

All plants were sub-irrigated with a nutrient solution containing 1.88 $g \cdot L^{-1}$ (277.5 ppm N) 15N-2.2P-12.5K (Peters 15-5-15 Ca-Mg Special, The Scotts Company, Marysville, OH, USA) as needed, maintaining electrical conductivity of 2.0 $dS \cdot m^{-1}$ and pH of 6.0. Plant canopy temperatures were recorded and maintained at 25.3/22.0ºC day/night. Mechanical mini fans (LS1225A-X, AC Infinity, City of Industry, CA, USA) were used to circulate the air to achieve uniform temperatures across treatments. All plants were harvested when plant height reached about 25 cm, which was at 19 days after treatment (DAT) and 40 days after sowing (DAS).

Table 20. Spectral characteristics of ten light quality treatments comprised of blue (B, 400-499 nm), green (G, 500-599 nm), red (R, 600-699nm), and far-red (FR, 700-780 nm) light.

Treatment	Single-band Photon Flux Density ($\mu mol \cdot m^{-2} \cdot s^{-1}$)			
	B	G	R	FR
$R_{53}B_{47}$[z]	106	-	119	3
$R_{47}B_{40}FR_{13}$	91	-	108	31
$R_{80}B_{20}$	44	-	181	4
$R_{66}B_{21}FR_{13}$	48	-	151	30
$R_{91}B_{9}$	21	-	205	3
$R_{80}B_{7}FR_{13}$	17	-	182	30
$R_{33}G_{40}B_{24}FR_{3}$	56	91	75	7
$R_{27}G_{33}B_{20}FR_{20}$	46	75	63	46
$R_{42}G_{43}B_{12}FR_{3}$	28	98	96	8
$R_{36}G_{37}B_{10}FR_{17}$	24	84	83	38
	Radiation Ratio			
	R:B	R:FR	B:FR	B:G
$R_{53}B_{47}$	1.12	-	-	-
$R_{47}B_{40}FR_{13}$	1.19	3.48	2.94	-
$R_{80}B_{20}$	4.11	-	-	-
$R_{66}B_{21}FR_{13}$	3.15	5.00	1.60	-
$R_{91}B_{9}$	9.76	-	-	-
$R_{80}B_{7}FR_{13}$	10.71	6.07	0.57	-
$R_{33}G_{40}B_{24}FR_{3}$	1.34	10.71	8.00	0.62
$R_{27}G_{33}B_{20}FR_{20}$	1.37	1.37	1.00	0.61
$R_{42}G_{43}B_{12}FR_{3}$	3.43	12.00	3.50	0.29
$R_{36}G_{37}B_{10}FR_{17}$	3.46	2.18	0.63	0.29
	Integrated Photon Flux Density ($\mu mol \cdot m^{-2} \cdot s^{-1}$)			PPE[y]
	YPFD[y]	PPFD[x]	TPFD[x]	
$R_{53}B_{47}$	188	225	228	0.80
$R_{47}B_{40}FR_{13}$	175	199	230	0.79
$R_{80}B_{20}$	210	225	229	0.87
$R_{66}B_{21}FR_{13}$	178	199	229	0.83
$R_{91}B_{9}$	220	226	229	0.89
$R_{80}B_{7}FR_{13}$	193	199	229	0.86
$R_{33}G_{40}B_{24}FR_{3}$	179	222	229	0.79
$R_{27}G_{33}B_{20}FR_{20}$	157	184	230	0.75
$R_{42}G_{43}B_{12}FR_{3}$	199	222	230	0.83
$R_{36}G_{37}B_{10}FR_{17}$	180	191	229	0.78

[z] Numbers indicate the percentage of R, B, G, and FR light in the total light intensity.
[y] Estimated phytochrome photoequilibrium (PPE) and yield photon flux density (YPFD, product of photo flux density and relative quantum efficiency) were calculated by the SpectraWiz software.
[x] Photon flux density (PPFD, 400-700 nm) and total photon flux density (TPFD, 400-780 nm) were measured using a PS-100 spectroradiometer and calculated accordingly.

6.3.3 Measurements

1. Photosynthesis and chlorophyll content

A portable gas exchange analyzer (CIRAS-3, PP Systems International, Amesbury, MA, USA) was used to measure the gas exchange rate of plant leaves at harvest. A PLC3 leaf cuvette with LED light unit was used, and light intensity, relative air humidity, and CO_2 concentration inside the leaf chamber were kept constant at 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 50%, and 390 $\mu mol \cdot mol^{-1}$, respectively. The third pair of leaves from the top was used for measuring gas exchange rate in green basil leaves. All measurements were taken until the net photosynthetic rate in basil leaves reached a steady state.

Soil plant analysis development (SPAD) index of basil was recorded weekly to quantify relative chlorophyll content in basil leaves using a chlorophyll meter SPAD-502 (Konica-Minolta cooperation, Ltd., Osaka, Japan). At harvest, approximately 0.2 g of basil leaves were cut into small pieces, then extracted in 80% methanol (v:v) for three days. The absorbance of extracts was measured at 663 nm and 645 nm using a spectrophotometer (Genesys 10S UV/Vis, Thermo Fisher Scientific, Madison, WI, USA), and the concentrations of chlorophyll a and chlorophyll b were calculated according to Porra et al. (1989). Chlorophyll a+b concentration was calculated accordingly.

2. Growth characteristics

Growth characteristics such as plant height, two perpendicular widths, and the number of internodes were recorded at harvest. Five plants per treatment were randomly selected for measurement. Leaf area was measured using a leaf area meter (LI-3100, LI-COR,

Lincoln, NE, USA), and shoot and root FW were recorded at harvest. The shoot and root tissues were dried at 80ºC in a drying oven (Grieve, Round Lake, IL, USA) for 3 days to determine the dry weight (DW).

3. Nutritional quality measurement

Five uniform plants were randomly selected for the measurements of concentrations of anthocyanin, phenolics, and flavonoids, and antioxidant capacity at harvest. Fresh basil leaves were collected in a cooler and immediately stored in a deep freezer (IU1786A, Thermo Fisher Scientific, Marietta, OH, USA) at -80ºC until phytochemical analyses.

Extraction. Approximately 2 g fresh plant leaves were ground in liquid nitrogen and extracted with 15 mL 1% acidified methanol at 4ºC in darkness. After overnight extraction, the mixture was centrifuged (Sorvall RC 6 Plus Centrifuge, Thermo Fisher Scientific, Madison, WI, USA) at 13,200 rpm (26,669 ×*g*) for 15 min, and the supernatant was collected for phytochemical analyses (Xu and Mou, 2016).

Anthocyanin analysis. The absorbance of extracts was measured at 530 nm using the aforementioned spectrophotometer, and the anthocyanin concentration was expressed as mg cyanidin-3-glucoside equivalent per 100 g FW of basil leaves using a molar extinction coefficient of 29,600 (Connor et al., 2002). Since the extracts were freshly prepared from leaf tissues maintained at -80ºC and did not undergo extensive processing or significant browning, a pH differential method for anthocyanin content was considered unnecessary (Connor et al., 2002).

Phenolics analysis. The total phenolics concentration of plant leaves was determined using the modified Folin-Ciocalteu reagent method described as the following: 100 μL

extraction sample was added to a mixture of 150 µL distilled water and 750 µL 1/10 dilution Folin-Ciocalteu reagent. After 6 min reaction, 600 µL 7.5% Na_2CO_3 was added and the mixture was incubated at 45°C in water bath for 10 min before the absorbance was measured at 725 nm using a microplate reader (ELx800, BioTek, Winooski, VT, USA). Results were expressed as mg of gallic acid equivalent per g FW of basil leaves (Xu and Mou, 2016).

Flavonoids analysis. The total flavonoid concentration of plant leaves was determined as the following: 20 µL extraction sample was added to a mixture of 85 µL distilled water and 5 µL 5% $NaNO_2$. After 6 min reaction, a 10 µL of 10% $AlCl_3 \cdot 6H_2O$ was added to the mixture. After another 5 min reaction, 35 µL of 1M NaOH and 20 µL distilled water were added to the mixture and the absorbance was measured at 520 nm using the aforementioned microplate reader (Dou et al., 2018). The results were expressed as mg of (+)-catechin hydrate equivalent per g FW of basil leaves.

Antioxidant capacity analysis. The antioxidant capacity of plant leaves was measured using the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) method (Arnao et al., 2001) described as the following: add a mixture of 150 µL basil leave extracts to 2.85 mL of $ABTS^+$ solution and incubate at room temperature for 10 min. The absorbance of mixed solution was measured at 734 nm using the aforementioned spectrophotometer. Antioxidant capacity of basil leaves was expressed as mg of Trolox equivalent antioxidant capacity per 100 g FW of basil leaves.

6.3.4 Statistical analysis

One-way analysis of variance (ANOVA) was conducted to analyze effects of light quality treatments on all measured parameters. Mean comparison among treatments was conducted using Student's t method. Correlation test was conducted using Pairwise Correlations method. All statistical analyses were performed using JMP (Version 13, SAS Institute Inc., Cary, NC, USA).

6.4 Results

6.4.1 Photosynthesis and chlorophyll content as influenced by far-red light substitution

Substituting far-red light for partial combined R&B light or white light did not affect the net photosynthetic rate (P_n) of basil leaves, while combined R&B LED light treatments increased P_n in basil leaves compare to white light (Fig. 23). SPAD reading of basil leaves decreased under treatments of substituting far-red light for partial PAR light in $R_{53}B_{47}$ and $R_{91}B_9$, while chlorophyll a+b concentration per leaf FW decreased under treatment of substituting far-red light for partial PAR light in $R_{91}B_{47}$ (Fig. 24A-B).

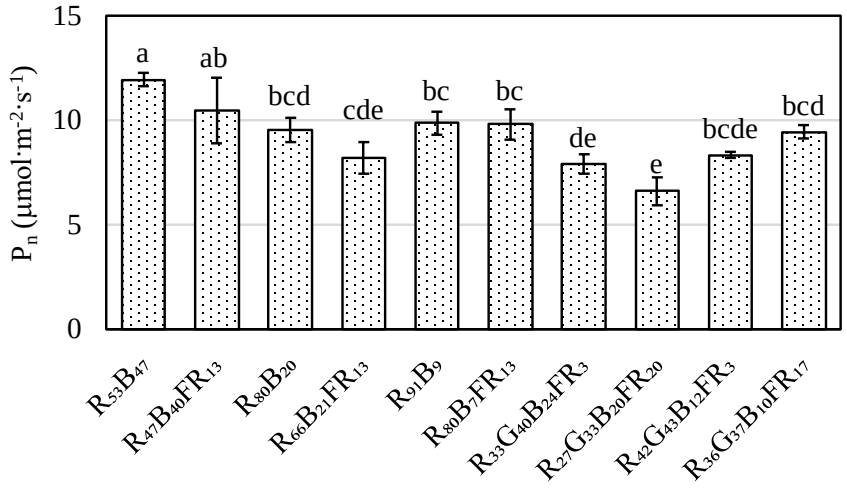

Figure 23. Net photosynthetic rate (P_n) of green basil 'Improved Genovese Compact' under different light quality treatments. Means followed by the same lowercase letters are not significantly different, according to Student's *t* mean comparison ($P < 0.05$). Bars represent standard errors.

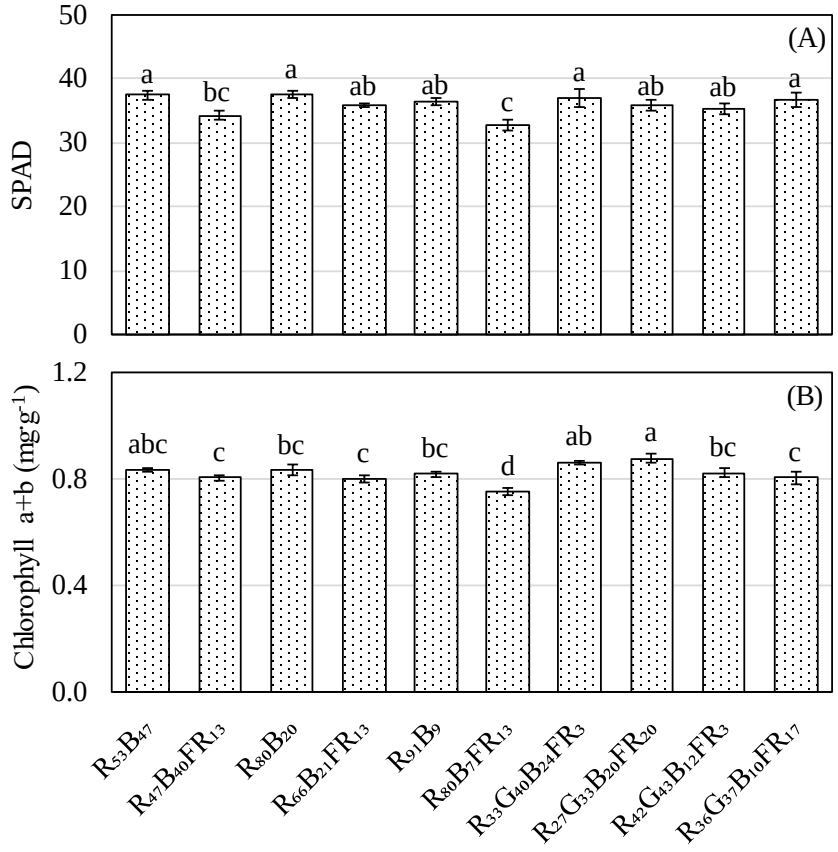

Figure 24. Soil plant analysis development (SPAD) (A) and chlorophyll a+b concentration (B) in green basil 'Improved Genovese Compact' under different light quality treatments. Means followed by the same lowercase letters are not significantly different, according to Student's *t* mean comparison ($P < 0.05$). Bars represent standard errors.

6.4.2 Plant growth and yield as influenced by far-red light substitution

Plant height and width in basil plants increased by 49%-65% and 10%-17%, respectively, by substituting far-red light for partial PAR light, except plant width under white LED ($R_{42}G_{43}B_{12}FR_3$) treatment (Fig. 25A-B). Substituting far-red light for partial PAR light increased leaf area by 12% in basil plants grown under $R_{91}B_9$ treatment but did not affect leaf area in the other treatments (Fig. 25C). Shoot FW and DW in basil plants

increased by 6%-23% and 4%-28%, respectively, by substituting far-red light for partial PAR light (Fig. 26A-B). Meanwhile, shoot FW was the lowest in basil plants grown under $R_{53}B_{47}$ and $R_{33}G_{40}B_{24}FR_3$ treatments among treatments without far-red light substitution.

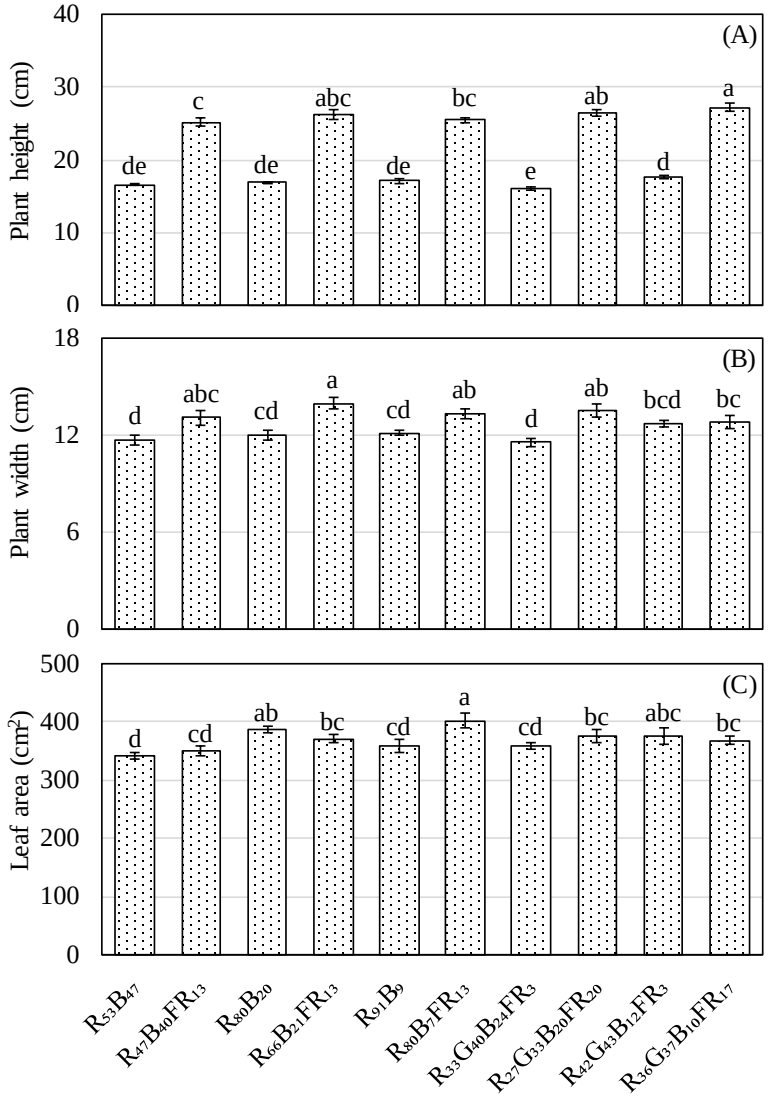

Figure 25. Plant height (A), plant width (B), and leaf area (C) in green basil 'Improved Genovese Compact' under different light quality treatments. Means followed by the same lowercase letters are not significantly different, according to Student's *t* mean comparison ($P < 0.05$). Bars represent standard errors.

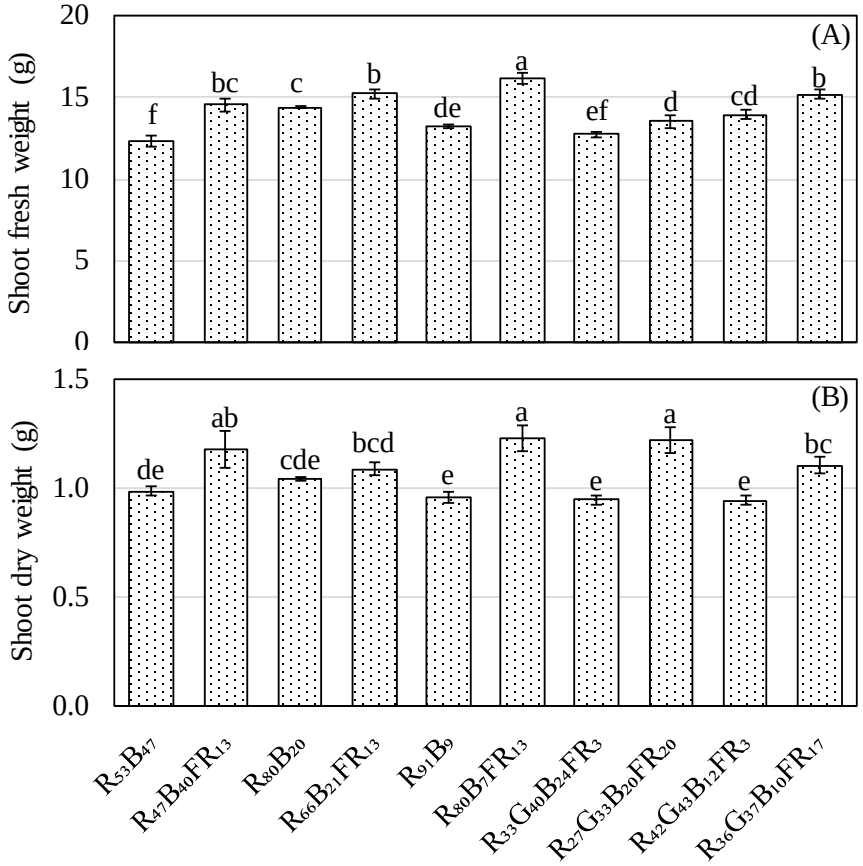

Figure 26. Shoot fresh weight (A) and shoot dry weight (B) in green basil 'Improved Genovese Compact' under different light quality treatments. Means followed by the same lowercase letters are not significantly different, according to Student's *t* mean comparison ($P < 0.05$). Bars represent standard errors.

6.4.3 Accumulation of secondary metabolites as influenced by far-red light substitution

Substituting far-red light for partial PAR light increased concentrations of anthocyanin, phenolics, and flavonoids in basil plants grown under $R_{80}B_{20}$ and $R_{42}G_{43}B_{12}FR_3$ treatments and tended to increase in plants grown under $R_{53}B_{47}$ and $R_{33}G_{40}B_{24}FR_3$ treatments, but differences were not significant (Fig. 27A-C). Consistently, substituting far-red light for partial PAR light increased antioxidant capacity by 21%, 44%, 22%, and 17% in basil

plants grown under $R_{53}B_{47}$, $R_{80}B_{20}$, $R_{33}G_{40}B_{24}FR_3$, and $R_{42}G_{43}B_{12}FR_3$ treatments, respectively, while showed no effects in plants grown under $R_{33}G_{40}B_{24}FR_3$ treatment (Fig. 27D).

The total amount of anthocyanin per plant increased by far-red light substitution under $R_{53}B_{47}$ treatment, while it was not affected under other treatments (Table 21). Under treatment $R_{80}B_{20}$, the total amount of phenolics and flavonoids per plant decreased and increased by far-red light substitution, respectively, while it was not affected under other treatments (Table 21). The antioxidant capacity per plant decreased by far-red light substitution under treatments $R_{53}B_{47}$, $R_{80}B_{20}$, and $R_{42}G_{43}B_{12}FR_3$, increased under treatment $R_{33}G_{40}B_{24}FR_3$, and was not affected under treatment $R_{91}B_9$ (Table 21).

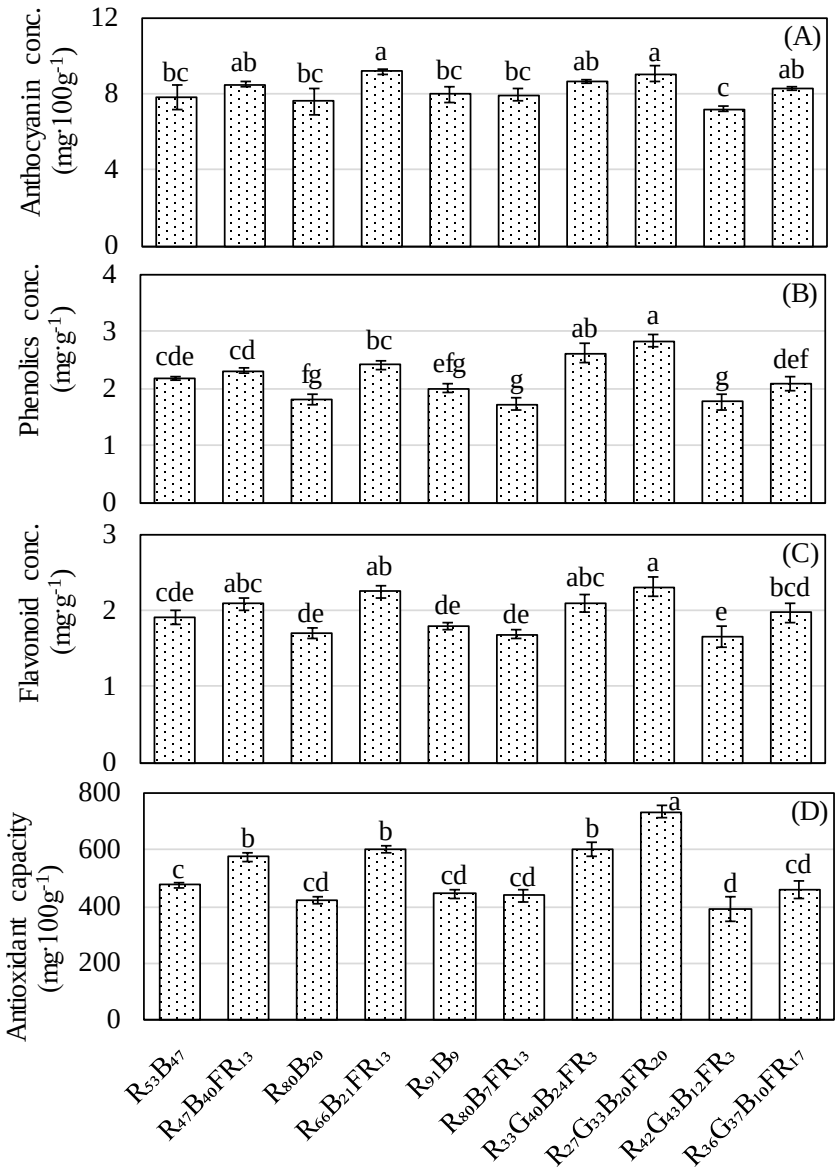

Figure 27. Anthocyanin concentration (conc.) (A), phenolics conc. (B), flavonoid conc. (C), and antioxidant capacity (D) in green basil 'Improved Genovese Compact' under different light quality treatments. Means followed by the same lowercase letters are not significantly different, according to Student's *t* mean comparison (*P* < 0.05). Bars represent standard errors.

Table 21. Total amount of anthocyanin, phenolics, flavonoids, and antioxidant capacity per plant in green basil 'Improved Genovese Compact' under different light quality treatments.

Treatments	Total Amount of Phytochemicals ($mg \cdot plant^{-1}$)							
	Anthocyanin		Phenolics		Flavonoids		Antioxidant Capacity	
$R_{53}B_{47}$	0.78	d[z]	21.8	cd	19.1	cde	47.5	c
$R_{47}B_{40}FR_{13}$	0.89	abc	24.3	bc	22.0	abc	60.3	b
$R_{80}B_{20}$	0.89	abcd	19.0	ab	17.8	e	49.1	c
$R_{66}B_{21}FR_{13}$	0.98	a	25.8	d	24.0	a	64.4	b
$R_{91}B_{9}$	0.86	bcd	21.4	cd	19.2	cde	47.6	c
$R_{80}B_{7}FR_{13}$	0.93	ab	20.2	d	19.7	bcde	51.3	c
$R_{33}G_{40}B_{24}FR_{3}$	0.90	abcd	27.0	ab	21.6	abcd	62.1	c
$R_{27}G_{33}B_{20}FR_{20}$	0.89	abcd	27.8	a	22.6	ab	71.9	a
$R_{42}G_{43}B_{12}FR_{3}$	0.80	cd	19.9	d	18.6	de	44.0	b
$R_{36}G_{37}B_{10}FR_{17}$	0.86	bcd	21.5	cd	20.3	bcde	47.3	c

[z] Means followed by the same lowercase letters are not significantly different, according to Student's *t* mean comparison ($P < 0.05$).

6.4.4 Correlations between growth parameters and YPFD and PPE

Correlations between growth parameters with YPFD and PPE were measured in two groups, treatments without far-red light substitution and treatments of substituting PAR light with far-red light. In treatments without far-red light substitution, no correlations between measured parameters and YPFD or PPE was observed (data not shown). In treatments of substituting PAR light with far-red light, chlorophyll a+b concentration and shoot FW of basil plants were negatively and positively correlated to YPFD and PPE, respectively (Fig. 28A-D).

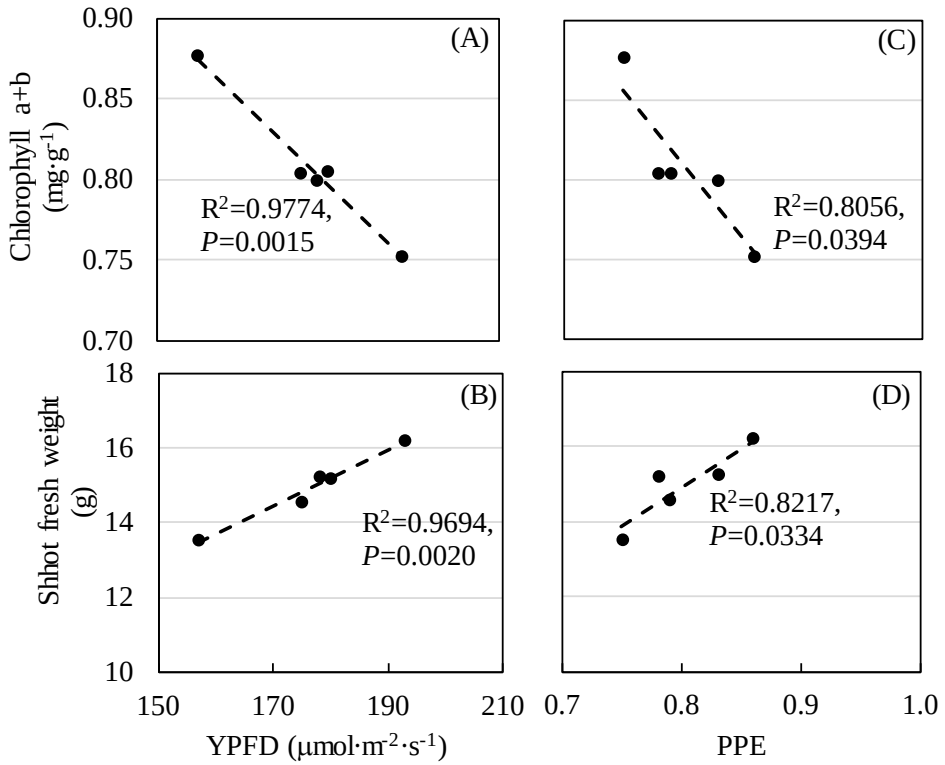

Figure 28. Correlations between yield photon flux density (YPFD) and chlorophyll a+b concentration per leaf fresh weight (A) and shoot fresh weight (B), and correlations between estimated phytochrome photoequilibrium (PPE) and chlorophyll a+b concentration per leaf fresh weight (C) and shoot fresh weight (D) in green basil 'Improved Genovese Compact' grown under different light quality treatments. Dash lines show regression between measured parameters and YPFD or PPE according to Pairwise Correlation method.

6.5 Discussion

6.5.1 Photosynthesis and chlorophyll content

The relative quantum efficiency of light wavelength dropped dramatically after 680 nm, which was 0.90 at 676 nm, 0.61 at 690 nm, but was only 0.04 at 750 nm (Sager et al., 1988). Therefore, it was hypothesized that substituting PAR light with far-red light would decrease plant photosynthesis due to decreased PPFD or YPFD. However, in the present

study, although P_n in basil leaves tended to decrease under far-red light treatments, the difference was not significant (Fig. 23). Similarly, P_n in white clover leaves (*Trifolium repens* 'Huia') was not affected by supplemental far-red light to PAR light, while chlorophyll content in treated leaves decreased (Heraut-Bron et al., 2000). We postulated that the excitation of two photosystems was better balanced by adding/substituting FR light to/for PAR light, which compensated reduced photosynthesis by decreased PPFD. This was evidenced by Zhen and Van Iersel (2017), who reported far-red light and PAR light had synergistic effects on photochemistry and photosynthesis. Another hypothesis was that under far-red light, a different localization of chloroplasts within cells could optimize the absorption of direct radiation, despite lower chlorophyll concentration or decreased PPFD (Heraut-Bron et al., 2000). However, P_n in 'Hokushin' cucumber (*Cucumis sativus*) plants decreased under treatment with lower R:FR ratio of 1.2 (metal-halide lamps) compared to treatment with higher R:FR ratio of 10.5 (white fluorescent lamps) at different PPFDs (Shibuya et al., 2012). Differences between the present study and Shibuya et al. (2012) study might be caused by different far-red light proportions. Although the far-red light proportion in Shibuya et al. (2012) study was not given, its R:FR ratio (1.2) was lower than the R:FR ratio used in the present study (1.37-10.67), indicating higher far-red light proportions used and resulted in decreased P_n by largely decreased PPFD or YPFD.

It was widely reported that far-red light substitution would decrease chlorophyll content in plant leaves as a result of investing resources in the most efficient way into plant expansion growth, to compensate for the adverse effects (Meng et al., 2019; Shibuya

et al., 2012). For example, chlorophyll concentration in ‘Rouxai’ lettuce plants decreased under treatments of substituting blue light with far-red light at same TPFD of 180 μmol·m^{-2}·s^{-1} (Meng et al., 2019). Similarly, in the present study, relative chlorophyll content and chlorophyll a+b concentration in basil plants both decreased under far-red light treatments, and chlorophyll a+b concentration was positively correlated to PPE under FR light treatments (Fig. 24A-B, 30C).

6.5.2 Plant growth and yield

As aforementioned, far-red light promotes plant expansion growth by triggering shade avoidance responses through photoreceptors (Demotes-Mainard et al., 2016). In general, plant expansion growth increases radiation interception and subsequently plant yield, which was confirmed in this study (Fig. 25, 26). Similarly, stem elongation and leaf expansion in geranium and snapdragon plants both increased under treatments of adding far-red light to combined R&B light and treatments of substituting R&B light with far-red light (Park and Runkle, 2017). Plant height, leaf length and width, and shoot FW and DW in lettuce and kale (*Brassica napus*) plants also increased under both treatments (supplemental far-red and substitution far-red light) (Li and Kubota, 2009; Meng et al., 2019).

Stimulation of stem elongation by low R:FR ratio is due to greater internode elongation rather than a greater number of internodes. The internode itself can perceive the R:FR environment and displays strong sensitivity and quick response to far-red, and perception of blue light by the leaves is necessary and enhances perception of R:FR by the internodes (Casal, 2013; Demotes-Mainard et al., 2016). In sunflower internodes, low R:FR ratio

induced high levels of two phytohormones (gibberellin, GA1 and auxin, IAA), supporting that these hormones act as growth-effectors in this process (Kurepin et al., 2007). Being different with stem elongation, leaf growth responses to R:FR ratios significantly varies, ranging from inhibition to promotion, which depends on the activity of phyB and phyD, whose mutants (*phyB* and *phyBphyD*) displayed a reduced leaf area (Casal and Smith, 1989; Demotes-Mainard et al., 2016). For example, leaf area in lettuce, kale, geranium, and snapdragon plants all increased under treatments of substituting PAR light with far-red light, while leaf area in petunia (*Petunia* × *hybrida*) and impatiens (*Impatiens walleriana*) plants were not affected (Meng et al., 2019; Park and Runkle, 2017). In the present study, leaf area in basil plants only increased under $R_{80}B_7FR_{13}$ treatment, while plant width increased under all far-red treatments except $R_{36}G_{37}B_{10}FR_{17}$ treatment (Fig. 25C), indicating petiole elongation was the major contributor to plant width promotion instead of leaf expansion. Effects of far-red light on leaf expansion is the balance between far-red light induced leaf expansion by triggering shade avoidance responses and reduced leaf expansion due to the resource competition with stem, or due to auxin-induced cytokinin breakdown in leaf primordia, resulting in reduced leaf cell proliferation (Demotes-Mainard et al., 2016). Compared to PPFD, YPFD has been suggested a more accurate predictor of plant photosynthesis and biomass accumulation, since photons of each wavelength are weighed by the relative quantum efficiency (Cope and Bugbee, 2013; Mccree, 1972). In the present study, although PPFD varies among far-red light treatments, it showed no relationship with growth parameters or plant yield, while YPFD was positively correlated to shoot FW (Fig. 28B).

6.5.3 Accumulation of secondary metabolites

Unexpectedly, phytochemical concentrations and antioxidant capacity in basil leaves increased or tended to increase under far-red light treatments while the total amount of phytochemicals per plant was mainly not affected (Fig. 27A-D, Table 21). Similarly, decreasing R:FR ratio increased anthocyanin content in 'Red Russian' kale seedlings, indicating far-red light could positively regulate anthocyanin accumulation in plants (Carvalho and Folta, 2014). However, most previous studies reported that enriched far-red light environments (low R:FR ratios) decrease the production of phytochemical in plants (Ballaré, 2014; Holopainen et al., 2018; Kadomura-Ishikawa et al., 2013). For example, rosmarinic acid concentration in basil and borage (*Borago officinalis*) plants showed a positive correlation with R:FR ratio (Schwend et al., 2016). Moreover, anthocyanin concentration in red leaf lettuce 'Outredgeous' was higher under combined R&B (PPE=0.73) and R&B&G (PPE=0.73) treatments, compared to plants grown under monochromatic red (PPE=0.72) and combined R&FR (PPE=0.53) treatments with the same PPFD of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$, indicating the presence of blue light was critical in regulating the synthesis of anthocyanin, instead of R:FR ratio or PPE (Stutte, 2009). We postulated the difference between studies was due to the great variability in the experimental setups, with inconsistencies in growing conditions such as spectral composition (inclusion or exclusion of green or blue light and different R:B ratios), PPFD values, or plant densities. Changes of phytochemical concentrations were not affected only by far-red light or R:FR ratios, but the coactions of red, blue, green, and far-red light. In the present study, substituting PAR light with far-red light not only changed R:FR ratio,

but also the R:B, B:G, and B:FR ratios. Also, the R:B ratios varies among far-red treatments in Schwend's et al. (2016) study.

6.6 Conclusion

Substituting PAR light with far-red light induced stem and petiole elongation in basil plants, resulting in greater shoot FW and DW, but showed no effects on leaf photosynthesis or leaf area. Meanwhile, substituting PAR light with far-red light resulted in increased concentrations of anthocyanin, phenolics, and flavonoids and antioxidant capacity in basil plants, which might be the coactions of changing proportions of red, blue, green, and far-red light. Far-red light substitution could be used as a tool to shorten plant production cycle as it accelerates plant growth rate.

CHAPTER VII

SUMMARY OF FINDINGS

With scenarios of increasing world population, resource (e.g. clean water, arable land) competition, and unusual climate/weather, there are increasing interests to produce culinary herbs and leafy greens in indoor vertical farms (IVFs), to supply fresh, local, and nutritious produce throughout the year. As one of the most important environmental factors, artificial lighting is one of the largest power consumption components in IVFs, in addition to altering plant photosynthesis, morphology, yield, and secondary metabolism. Therefore, to optimize the lighting environment in IVFs, we studied the effects of light quantity, light quality, and supplemental UV-B lighting on plant growth and development in the present study. The main effects of light environment on plant growth and yield are summarized in findings from (i) through (vi) and finding (vii) summarizes the effect of light environment on plant secondary metabolites accumulation.

(i) Photosynthetic capacity in green basil (*Ocimum basilicum* 'Improved Genovese Compact') plants increased under higher daily light integrals (DLIs) of 12.9, 16.5, and 17.8 $mol \cdot m^{-2} \cdot d^{-1}$, resulting in larger and thicker leaves, greater leaf and shoot yield, and higher dry matter content. No differences on shoot fresh weight in basil plants were observed among DLIs of 12.9, 16.5, and 17.8 $mol \cdot m^{-2} \cdot d^{-1}$.

(ii) Red light (600-699 nm) has the highest relative quantum efficiency (0.91) compared to other light spectra in the photosynthetically active radiation (PAR, 400-699 nm) wavelength. Higher red light proportions in the combined red and blue (R&B) light increased stem elongation and leaf area in basil 'Improved Genovese Compact' (green)

and ‘Red Rubin’ (purple), green mustard (*Brassica carinata* ‘Amara’), red mustard (*Brassica juncea* ‘Red Giant’), green kale (*Brassica napus pabularia* ‘Siberian’), and red kale (*Brassica oleracea* ‘Scarlet’) plants, and subsequently greater plant yield due to increased light interception.

(iii) Increases of blue light (400-599 nm) proportions in the combined R&B light increased or tended to increase net photosynthetic rate (P_n) in basil and kale plants, due to increased chlorophyll content and stomatal opening. Changes of blue light proportions showed no effects on plant photosynthesis in mustard plants. However, increases of blue light proportions decreased plant yield because blue light induced inhibition of stem elongation and leaf expansion (leaf area), indicating plant photomorphogenesis dominated biomass accumulation in basil and *Brassica* species instead of photosynthesis.

(iv) Addition of green light (400-499 nm) to the combined R&B light played a negative role or had no effects on P_n, chlorophyll content, leaf area, or plant yield. Green light effects on leaf expansion were minimal compared to red or blue light. However, substituting partial red or blue light with green light increased P_n in the lower leaves in purple basil plants because green light penetrates into deeper plant canopy and increased photosynthetic photon flux density (PPFD) in the lower level plant canopy. This indicated compactness of plant canopy would strengthen the positive effects of green light, which could potentially increase plant yield.

(v) Substituting PAR light with far-red light (700-780 nm) induced shade avoidance responses in green basil plants such as stem and petiole elongation, resulting in greater plant yield, but showed no effects on leaf photosynthesis or leaf area. With far-red light

substitution, yield photon flux density (product of photon flux density and relative quantum efficiency) has been suggested a more accurate predictor of plant photosynthesis and biomass accumulation compared to PPFD, since photons of each wavelength are weighed by the relative quantum efficiency.

(vi) Supplemental ultraviolet-B (UV-B) radiation decreased plant height, width, and leaf area in both green and purple basil plants, and the detriment increased with increasing UV-B radiation doses. Under higher UV-B radiation doses such as 1 $h \cdot d^{-1}$ or 2 $h \cdot d^{-1}$ for 5 days, basil plants also showed leaf bronzing, chlorosis, waxy appearance, and premature leaf defoliation. In *Brassica* species, lower UV-B radiation doses from 0.5 $h \cdot d^{-1}$ for 1 day to 1 $h \cdot d^{-1}$ for 3 days did not affect plant growth (e.g. plant height, width, leaf area) or yield, but decreased leaf photosynthesis.

(vii) Nutritional contents of green basil leaves (i.e. soluble sugar, anthocyanin, phenolics, and flavonoids) were positively correlated with DLI. All light spectrum, including red, blue, green, far-red, and UV-B lights are involved in the synthesis of secondary metabolites, and their effects are dependent on plant species and specific phytochemicals. Increases of blue light proportions in the combined R&B light enriched anthocyanin concentration in green kale and red mustard plants, but showed no effects in green or purple basil, red kale, or green mustard plants. Similarly, increases of blue light proportions enriched phenolic concentration in all tested plant species except red mustard plants, and increased flavonoid concentration in purple basil plants. However, the total amount of phytochemicals per plant decreased with increases of blue light proportions. Substituting partial red and/or blue light with green light decreased both concentrations

and total amount of phytochemicals in basil plants. Specifically, phytonutrients accumulation and antioxidant capacity in green basil plants decreased under treatments of substituting partial blue or R&B light with green light. Phytonutrients accumulation and antioxidant capacity in purple basil plants decreased under treatments of substituting partial red or R&B light with green light. This indicated blue and red light plays a major function in inducing the secondary metabolites accumulation in green and purple basil plants, respectively. Substituting PAR light with far-red light also resulted in increased concentrations of anthocyanin, phenolics, and flavonoids and antioxidant capacity in green basil plants, while the total amount of phytochemicals per plant was not affected. Supplemental UV-B radiation significantly improved concentrations of secondary metabolites and antioxidant capacity in green basil, green kale, red kale, and green mustard plants. Noticeably, supplemental UV-B radiation enhanced phytonutrients accumulation up to 169% in green basil plants. Antioxidant capacity in green basil, purple basil, green kale, red kale, and green mustard plants were all positively correlated with supplemental UV-B radiation doses, while not affected by UV-B treatments in red mustard plants. Meanwhile, effects of UV-B radiation on basil plants interacted with PPFD, which is, low PPFD increased plant sensitivity to UV-B radiation and UV-B radiation compensated for reduced accumulation of flavonoids in green basil plants grown under low PPFD.

In conclusion, a predominated red light with supplemental blue light and a small proportion of far-red light (13% in the present study) at a PPFD of 224 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 16-h photoperiod was suggested for basil and *Brassica* species production in IVFs. Meanwhile, supplemental UV-B radiation (at 16.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$) of 1 $h \cdot d^{-1}$ for 2-3 days

prior to harvest could enrich plant secondary metabolites accumulation without yield reduction.

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