Morphological and Physiological Responses in Basil and *Brassica* Species to Different Proportions of Red, Blue, and Green Wavelengths in Indoor Vertical Farming

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ABSTRACT. Understanding the responses of plant growth and secondary metabolite synthesis to different light wavelengths is important for optimizing lighting conditions for vegetable production in indoor vertical farms. Basil (*Ocimum basilicum*) ‘Improved Genovese Compact’ (green leaf) and ‘Red Rubin’ (purple leaf), green mustard ‘Amara’ (*Brassica carinata*), red mustard ‘Red Giant’ (*Brassica juncea*), green kale ‘Siberian’ (*Brassica napus* var. *pabularia*), and red kale ‘Scarlet’ (*Brassica oleracea*), which are high-value and multifunctional culinary herbs and leafy greens, were used to characterize the effects of red (R), blue (B), and green (G) wavelengths on plant photosynthesis, morphology, biomass production, and secondary metabolites accumulation. Light quality treatments consisted of three R and B light combinations, R₈₈B₁₂ (the proportions of R and B wavelengths were 88% and 12%, respectively), R₇₆B₂₄, and R₅₁B₄₉, and two white light combinations, R₄₄B₁₂G₄₄ (the proportions of R, B, and G wavelengths were 44%, 12%, and 44%, respectively) and R₃₅B₂₄G₄₁. Experiments were conducted in a walk-in growth room with a photosynthetic photon flux density set at 224 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and a 16-hour photoperiod. Results indicated that the net photosynthesis in purple basil and green kale were positively correlated with B proportions (BP), and that higher BP increased the relative chlorophyll concentration in purple basil and red kale. In contrast, higher BP suppressed stem elongation and leaf expansion and reduced shoot biomass in all tested species except red mustard. Higher BP increased phytochemical concentrations but decreased the total amounts of phytochemicals per plant. For all basil and brassica (*Brassica* sp.) cultivars, the inclusion of G wavelengths decreased shoot biomass compared with that of plants grown under R and B light combinations with similar BP. Inclusion of G wavelengths stimulated stem elongation in green basil and green mustard under 12% BP; whereas it suppressed stem elongation in purple basil, green kale, red kale, and green mustard under 24% BP. The effects on phytochemical accumulation were species-specific for the inclusion of G wavelengths. Considering biomass production, nutritional values, and working environment for growers, a white light with lower BP and G proportions is recommended for culinary herbs and *Brassica* leafy greens production at vertical farms.

In recent years, food production under controlled environments, especially at vertical farms (VF), has been gaining attention due to the increasing world population, urbanization, global climate change, competition for resources (e.g., land, water, and energy), and increasing demand for locally produced

fresh food with high quality (Despommier, 2013; Kozai et al., 2015; Tornaghi, 2017). Because electricity for powering electrical lighting is one of the largest energy consumption components of VF, optimizing lighting is of great importance. However, lighting conditions significantly affect plant growth, development, and secondary metabolism (Dou et al., 2017, 2018, 2019a, 2019b; Hosseini et al., 2018; Roupael et al., 2018). With the advancement of light-emitting diode (LED) technology and its customizable nature, the effects of different light wavelengths on plant growth and secondary metabolites accumulation can be characterized (Darko et al., 2014; Mitchell et al., 2015; Ouzounis et al., 2015; Stutte, 2009), thus providing valuable and practical information for the optimization of lighting environments at VF.

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Photosynthetically active radiation [*PAR* (400–700 nm)] including blue [B (400–499 nm)], green [G (500–599 nm)], and red [R (600–699 nm)] wavelengths designates the spectral range providing light energy for photosynthetic organisms, thus affecting the plant biomass accumulation (Kozai et al., 2015). Within the *PAR* range, R and B wavelengths have been studied mainly because of their high absorptivity in the absorption spectra of chlorophyll, with a strong positive correlation between chlorophyll absorption and photosynthetic efficiency (Kopsell et al., 2014; McCree, 1972). In general, a red and blue (R&B) light combination with a high R:B ratio achieves greater plant yield (biomass production) compared with monochromatic R or B wavelengths (Fan et al., 2013; Li, 2010; Ohashi-Kaneko et al., 2007; Wollaeger and Runkle, 2014). However, the optimal R:B ratio or R proportion/B proportion (RP/BP) in R&B light combinations for plant growth and biomass accumulation is still unknown and may be crop-specific and cultivar-specific. For instance, Snowden et al. (2016) reported that crop yield was negatively related to or unaffected by BP. Specifically, dry weight (DW) in tomato (*Solanum lycopersicum*), cucumber (*Cucumis sativus*), radish (*Raphanus sativus*), and pepper (*Capsicum annuum*) decreased when BP increased from 11% to 28%, whereas DW in soybean (*Glycine max*), lettuce (*Lactuca sativa*), and wheat (*Triticum aestivum*) was unaffected by changing BP (Snowden et al., 2016). Similarly, the shoot fresh weight (FW) in lettuce and kale (*Brassica oleracea*) decreased as BP increased from 0% to 33% (Meng et al., 2019). In contrast, shoot FW of basil was highest under 59% BP compared with plants grown under 48%, 40%, and 15% BP, indicating that crop yield may be positively influenced by BP (Piovene et al., 2015).

It was reported that R and B wavelengths influence the biosynthesis of secondary metabolites in plants, particularly phenolics, flavonoids, and anthocyanins. These secondary metabolites have important roles as antioxidants scavenging reactive oxygen species, ultraviolet screening molecules, or anti-infection agents (Klem et al., 2019; Logan et al., 2015). For example, the concentration of rosmarinic acid, the major phenolic acid in basil, was twice that in plants grown under R and white (W) wavelengths compared with plants grown under B wavelengths, whereas the concentration of chicoric acid, the second major phenolic acid in basil (*Ocimum basilicum*), was higher under B wavelengths than under R wavelengths (Amaki et al., 2011; Shiga et al., 2009; Shoji et al., 2011). Qian et al. (2016) reported that the maximum content of vitamin C in kale sprouts was detected under W light, whereas the highest levels of total phenolics and anthocyanins as well as the strongest antioxidant capacity were observed under B wavelengths. Similarly, the l-menthol content in Japanese mint (*Mentha arvensis*) was enhanced by R wavelengths, whereas polyphenol, total antioxidants, anthocyanin, and carotenoid concentration in leaf lettuce and β -carotene and lutein concentrations in spinach (*Spinacia oleracea*) were increased by B wavelengths (Johkan et al., 2010; Li and Kubota, 2009; Nishioka et al., 2008). Based on previous research, the effects of R and B wavelengths on secondary metabolism vary among plant species and phytochemical compounds, and more research is needed to determine the balance between R and B wavelengths to achieve greater yield and higher nutritional quality for plant production in VF.

Compared with R and B wavelengths, G wavelengths are studied less due to their low absorptivity in the absorption

spectra of chlorophyll (Kopsell et al., 2014), but the effects of G wavelengths on plant growth are gaining more attention from researchers (Dou et al., 2019a; Folta and Maruhnich, 2007; Terashima et al., 2009; Wang and Folta, 2013; Zhang and Folta, 2012). At the leaf scale or at the whole plant canopy level, the relative quantum efficiency of broadband G wavelengths is 0.87, which is slightly lower than that of R wavelengths (0.91) but higher than that of B wavelengths (0.73) (Sager et al., 1988). Although R and B wavelengths are strongly absorbed by the upper plant canopy, G wavelengths penetrate deeper in the plant canopy, which could potentially increase plant photosynthesis in lower plant canopy levels and subsequently increase plant yield at the whole plant canopy level (Dou et al., 2019a; Terashima et al., 2009; Wang and Folta, 2013). In addition, G wavelengths induce shade avoidance responses in plants, such as increased plant height and leaf area, which may increase light interception by a larger plant canopy (Zhang and Folta, 2012). In fact, at a photosynthetic photon flux density (*PPFD*) of 150 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with a 18-h photoperiod, the leaf area and shoot FW and DW in ‘Waldmann’s Green’ lettuce grown under R₆₁B₁₅G₂₄ [RP, BP, and G proportion (GP) at 61%, 15%, and 24%, respectively] increased by 31%, 45%, and 47%, respectively, compared with plants grown under R₈₄B₁₆ (Kim et al., 2004). Moreover, the inclusion of G wavelengths in growth light sources would make plants appear a normal green color instead of purplish, which makes the visual assessment of physiological disorders easier and offers psychological benefits to farm workers. However, other studies reported that G wavelengths can reverse B or R wavelength-induced responses, such as B wavelength-induced stomatal opening and pigment formation, which negatively affect plant photosynthesis or growth (Folta and Maruhnich, 2007; Talbott et al., 2006). These contrasting results cause the inclusion of G wavelengths in growth light to be an ongoing discussion.

Basil and *Brassica* leafy greens are highly diverse in species and cultivars and are a valuable component of the human diet because of their relatively high levels of bioactive secondary metabolites (Keservani et al., 2010; Makri and Kintzios, 2008; Qian et al., 2016). There have been many studies focused on the effects of R and B wavelengths or the effects of G wavelengths on plant growth, but extensive research regarding plant responses to changing RP, BP, and GP together is still required, especially for different plant species and cultivars. Based on previous research, we chose three R&B light combinations with higher R:B ratios and two W light (inclusion of G wavelengths to R&B wavelengths) to investigate the effects of different RP, BP, and GP on photosynthesis, morphology, yield, and secondary metabolism in basil and *Brassica* species (model plants) in VF. Specifically, we aimed to: 1) determine the optimal R:B ratio for green and purple/red leaf basil and *Brassica* leafy greens production; and 2) characterize if inclusion of G wavelengths will increase plant biomass production and alter plant secondary metabolism. These study findings should provide a theoretical basis for the definition of optimal light quality for high yield and improved nutritional quality production of culinary herbs and *Brassica* leafy greens cultivated at VF.

Materials and Methods

Plant materials and growing conditions

All experiments were conducted in a walk-in growth room (35 m²) with vertical grow racks at Texas A&M AgriLife

Research Center in El Paso using basil (green leaf ‘Improved Genovese Compact’ and purple leaf ‘Red Rubin’), mustard [green leaf ‘Amara’ (*Brassica carinata*) and red leaf ‘Red Giant’ (*Brassica juncea*)], and kale [green leaf ‘Siberian’ (*Brassica napus* var. *pabularia*) and red leaf ‘Scarlet’ (*B. oleracea*)] (Johnny’s Selected Seeds, Winslow, ME). Experiments for multiple species were conducted during separate time periods, and the treatment time was different for each cultivar due to different growth rate. The experiments for green and purple basil were conducted twice per cultivar, and experiments for mustard and kale were conducted one time per cultivar. Green and purple basil were harvested at 21 and 28 d after treatment (DAT), respectively, which were at 42 and 49 d after sowing (DAS), respectively. Green and red mustard were both harvested at 21 DAT (35 DAS). Green and red kale were harvested at 18 and 25 DAT (32 and 39 DAS), respectively. All plants were harvested when the plant height reached ≈ 25 cm.

For all experiments, one seed per cell was sown in 72-square-cell trays (cell size, 3.86 cm length $\times$ 5.72 cm height; volume, 59 cm³) with substrate (peatmoss 41%, vermiculite 34%, pine bark 25%; Metro-Mix 360; Sun Gro Horticulture, Bellevue, WA). All trays were placed under intermittent mist (15 s every 30 min) in a polycarbonate greenhouse for germination. Seedlings were moved from the mist after emergence of cotyledon and grown in a greenhouse until transplanting. The average temperatures and relative humidity in the greenhouse for green leaf basil, purple leaf basil, mustard, and kale were as follows: $28.2 \pm 2.8/18.1 \pm 3.9$ °C and $28\% \pm 6\%/38\% \pm 7\%$; $24.0 \pm 3.6/16.3 \pm 3.2$ °C and $34\% \pm 10\%/52\% \pm 10\%$; $25.9 \pm 3.0/19.4 \pm 1.3$ °C and $18\% \pm 5\%/36\% \pm 7\%$; and $25.6 \pm 3.0/19.8 \pm 1.6$ °C and $20\% \pm 8\%/33\% \pm 6\%$ (day/night), respectively. For each cultivar, 150 seedlings with one pair of true leaves expanded were transplanted to grow pots (pot size, 9.52 cm length $\times$ 8.26 cm height; volume, 574 cm³) with substrate. Uniform plants were selected and moved to the walk-in growth room to initiate the different light quality treatments. There were 18, 12, and 12 plants per treatment per cultivar for basil, kale, and mustard, respectively. For five light quality treatment, two vertical grow racks with four shelves per grow rack were used, and each shelf was designated as one light treatment.

After transplanting, all plants were sub-irrigated manually with a nutrient solution containing $1.88 \text{ g} \cdot \text{L}^{-1}$ 15N–2.2P–12.5K water-soluble fertilizer (Peters 15–5–15 Ca-Mg Special; Scotts Co., Marysville, OH) as needed. The nutrient solution was mixed and stored in a 378-L tank with lid, and the electrical conductivity (EC) was $2.01 \pm 0.06 \text{ dS} \cdot \text{m}^{-1}$ throughout the experiment, which was measured using an EC/pH meter (model B-173; Horiba, Kyoto, Japan). The pH of the nutrient solution was measured using the aforementioned EC/pH meter and adjusted to 5.98 ± 0.03 using potassium hydroxide. Planting density for all cultivars was 79 plants/m². The air temperature in the growth room was set at 24/21 °C (day/night) for basil, mustard, and kale. The relative humidity and CO₂ concentration were not controlled in the growth room. The plant canopy temperature of each treatment was recorded every 30 min using type-T thermocouples connected to a datalogger (CR1000; Campbell Scientific, Logan, UT). The average plant canopy temperatures during the experiment were $23.5 \pm 0.25/21.3 \pm 0.20$, $23.4 \pm 0.31/20.2 \pm 0.17$, and $22.5 \pm 0.23/20.0 \pm 0.20$ °C (day/night) for basil, mustard, and kale, respectively. Mechanical mini fans (LS1225A-X; AC Infinity, City of Industry, CA)

were used for air circulation to achieve uniform temperatures across treatments.

Light quality treatments

A total of five light quality treatments were used: three R&B LED light combinations with different BP and two W light (inclusion of G wavelengths). The three R&B light combinations consisted of R₈₈B₁₂ (model GEHL48HPPR; GE Current, Boston, MA), R₇₆B₂₄ (model GEHL48HPPB; GE Current), and R₅₁B₄₉ (model GEHL48HPPV; GE Current). The two W light treatments consisted of R₄₄B₁₂G₄₄ (W LED; model GEHL48HWTB; GE Current) and R₃₅B₂₄G₄₁ (W fluorescent light; cool White Alto Linear; Philips Lighting, Somerset, NJ). All treatments were maintained at the same PPFD level of $224 \pm 12 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ with a 16-h photoperiod from 0600 to 2200 HR. Light spectral distribution (Fig. 1) and PPFD were measured at 15 cm underneath the light source at nine spots for each treatment using a PS-100 spectroradiometer (Apogee Instruments, Logan, UT) before placing plants. To minimize disproportionate light distribution within each treatment, all plants were rearranged systematically every 3 d.

Measurements

GAS EXCHANGE RATE AND RELATIVE CHLOROPHYLL CONTENT.

A portable gas exchange analyzer (CIRAS-3; PP Systems, Amesbury, MA) was used to measure the comparative net photosynthesis (P_n) of plant leaves at harvest. A leaf cuvette (PLC3; PP Systems) with an LED light unit (W light, in which RP, BP, and GP were 38%, 25%, and 37%, respectively) was used, and PPFD, relative air humidity, and CO₂ concentration inside the leaf chamber were kept constant at $800 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, 50%, and $390 \mu\text{mol} \cdot \text{mol}^{-1}$, respectively. The third leaf from the top (the uppermost fully expanded leaf) was used for measurements. Measurements were obtained when P_n reached a steady state.

The soil plant analysis development (SPAD) index of basil leaves was measured at harvest to quantify relative chlorophyll content using a chlorophyll meter (SPAD-502; Konica-Minolta, Osaka, Japan). The third leaf from the top was used for

Fig. 1. Spectral distribution of different light quality treatments including three combined red (R) and blue (B) light combinations, R₈₈B₁₂ (the proportions of R and B wavelengths were 88% and 12%, respectively), R₇₆B₂₄ (the proportions of R and B wavelengths were 76% and 24%, respectively), and R₅₁B₄₉ (the proportions of R and B wavelengths were 51% and 49%, respectively), and two white light combinations, R₄₄B₁₂G₄₄ [white light-emitting diode with R, B, and green (G) wavelength proportions of 44%, 12%, and 44%, respectively] and R₃₅B₂₄G₄₁ (white fluorescent light with R, B, and G wavelength proportions of 35%, 24%, and 41%, respectively). Photosynthetic photon flux density (PPFD) and spectral distribution were measured using a spectroradiometer (PS-100; Apogee Instruments, Logan, UT).

measurements. Three measurements were obtained for each plant and the average was recorded for data analysis.

GROWTH PARAMETERS AND YIELD. Growth characteristics including plant height and leaf area were recorded at harvest. Plant yield including shoot FW and DW were also measured at the end of the experiment. Leaf area was measured using a leaf area meter (LI-3100; LI-COR, Lincoln, NE). Shoot tissues were dried at 80 °C in a drying oven (Grieve, Round Lake, IL) for 3 d to determine the shoot DW. The specific leaf area (SLA) was calculated as the ratio of the leaf area to the leaf DW.

SECONDARY PLANT METABOLITES. Extraction. Fresh leaves were collected in a cooler and immediately stored in a deep freezer (IU1786A; Thermo Fisher Scientific, Waltham, MA) at –80 °C until phytochemical analyses. Approximately 2 g of fresh 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; Thermo Fisher Scientific) at 26,669 g_n for 15 min, and the supernatant was collected for phytochemical analyses (Xu and Mou, 2016).

Anthocyanin analysis. Absorbance of the extract collected from extraction was measured at 530 nm vs. acidified methanol (1%) blank using a spectrophotometer (Genesys 10S ultraviolet/Vis; Thermo Fisher Scientific). Because the extracts were prepared from leaf tissues maintained at –80 °C and did not undergo extensive processing or significant browning, a pH differential method for anthocyanin content was considered unnecessary (Connor et al., 2002). Anthocyanin concentration was expressed as milligrams of cyanidin-3-glucoside equivalent per 100 g FW of plant leaves using a molar extinction coefficient of 29,600, which was calculated as follows (Connor et al., 2002):

$$\text{Anthocyanin concentration (mg/100 g FW)} = \frac{V \times n \times M \times A \times 100}{\epsilon \times m}$$

where V is the volume of extracted liquid (milliliters), n is the dilution factor, M is the molecular weight of cyaniding-3-glucoside (449.2), A is the absorbance at 530 nm, ϵ is the molar extinction coefficient (29,600), and m is the weight of the sample.

Phenolics analysis. The total phenolics concentration of plant leaves was determined using the modified Folin-Ciocalteu reagent method (Xu and Mou, 2016) as follows: 100- μ L extraction sample was added to a mixture of 150 μ L distilled water and 750 μ L 10% dilution Folin-Ciocalteu reagent. After a 6-min reaction, 600 μ L 7.5% Na_2CO_3 was added and 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). Results were expressed as milligrams of gallic acid equivalent per gram FW of plant leaves.

Flavonoids analysis. The total flavonoid concentration of plant leaves was determined (Dou et al., 2018) as follows: 20- μ L extraction sample was added to a mixture of 85 μ L distilled water and 5 μ L 5% NaNO_2 . After a 6-min reaction, 10 μ L of 10% $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ was added to the mixture. After another 5-min reaction, 35 μ L of 1 M NaOH and 20 μ L distilled water were added to the mixture and absorbance was measured at 520 nm using the aforementioned microplate reader. Results were expressed as milligrams (+)-catechin hydrate equivalent per gram FW of plant leaves.

Antioxidant capacity analysis. The antioxidant capacity of plant leaves was measured using the 2,2'-azino-bis(3-ethyl-benzothiazoline-6-sulphonic acid) (ABTS) method (Arnao et al., 2001) as follows: a mixture of 150 μ L of basil leave extract was added to 2.85 mL of colored free radical ABTS (ABTS^+) solution and incubated at room temperature for 10 min. Absorbance of the mixture was measured at 734 nm using the aforementioned spectrophotometer. The antioxidant capacity was expressed as milligrams of Trolox equivalent antioxidant capacity per 100 g FW of plant leaves.

Statistical analysis

The experiment was designed as a completely randomized design to test the effects of light quality (changing RP, BP, and GP) on all measured parameters. Five plants per cultivar per treatment were selected for each measurement. One-way analysis of variance was conducted and a mean comparison among treatments was conducted using Tukey's honestly significant difference test ($P \leq 0.05$). A correlation test was conducted using a pairwise correlations method, and the correlation coefficient γ is presented when a correlation is significant ($P \leq 0.05$). All statistical analyses were performed using JMP software (version 13; SAS Institute, Cary, NC).

Results

Morphological and physiological responses in basil to different RP, BP, and GP

Light quality showed no effects on comparative P_n or SPAD of green basil (Figs. 2 and 3). In purple basil, comparative P_n under $R_{51}B_{49}$ was 15% to 34% higher compared with plants grown under other treatments (Fig. 2) and was positively correlated with BP with a coefficient of 0.9107. Similarly, the SPAD reading of purple basil was highest under $R_{51}B_{49}$ and $R_{76}B_{24}$ (Fig. 3). The inclusion of G wavelengths showed no effect on comparative P_n in purple basil compared with plants grown under R&B light combinations with similar BP (Fig. 2).

Fig. 2. Net photosynthesis of green basil 'Improved Genovese Compact', purple basil 'Red Rubin', green mustard 'Amara', red mustard 'Red Giant', green kale 'Siberian', and red kale 'Scarlet' grown under five light quality treatments: three combined red (R) and blue (B) light combinations, $R_{88}B_{12}$ (the proportions of R and B wavelengths were 88% and 12%, respectively), $R_{76}B_{24}$ (the proportions of R and B wavelength were 76% and 24%, respectively), and $R_{51}B_{49}$ (the proportions of R and B wavelengths were 51% and 49%, respectively) and two white light combinations, $R_{44}B_{12}G_{44}$ [white light-emitting diode with R, B, and green (G) wavelength proportions of 44%, 12%, and 44%, respectively] and $R_{35}B_{24}G_{41}$ (white fluorescent light with R, B, and G wavelength proportions of 35%, 24%, and 41%, respectively). Means followed by different lowercase letters indicate a significant difference according to one-way analysis of variance ($P \leq 0.05$).

Fig. 3. Soil plant analysis development (SPAD) measurements of green basil 'Improved Genovese Compact', purple basil 'Red Rubin', green mustard 'Amara', red mustard 'Red Giant', green kale 'Siberian', and red kale 'Scarlet' grown under three combined red (R) and blue (B) light combinations, R₈₈B₁₂ (the proportions of R and B wavelengths were 88% and 12%, respectively), R₇₆B₂₄ (the proportions of R and B wavelengths were 76% and 24%, respectively), and R₅₁B₄₉ (the proportions of R and B wavelengths were 51% and 49%, respectively), and two white light combinations, R₄₄B₁₂G₄₄ [white light-emitting diode with R, B, and green (G) wavelength proportions of 44%, 12%, and 44%, respectively] and R₃₅B₂₄G₄₁ (white fluorescent light with R, B, and G wavelength proportions of 35%, 24%, and 41%, respectively). Means followed by different lowercase letters indicate a significant difference according to one-way analysis of variance ($P \leq 0.05$).

However, the inclusion of G wavelengths decreased SPAD in purple basil by 11% and 9% compared with plants grown under R&B light combinations with 12% and 24% BP, respectively (Fig. 3).

The plant height and leaf area of green basil were both lowest under treatments R₅₁B₄₉ and R₇₆B₂₄ among the three R&B light combinations (Fig. 4A). The plant height and leaf area of purple basil were both lowest under treatment R₅₁B₄₉ among the three R&B light combinations (Fig. 4B). Under 12% BP, the inclusion of G wavelengths increased the plant height of green basil, but it showed no effects on purple basil. In contrast, under 24% BP, the inclusion of G wavelengths showed no effects on plant height of green basil, but it decreased the plant height of purple basil (Fig. 4A and B). The leaf area of green basil tended to decrease by the inclusion of G wavelengths, but the difference was not significant. The leaf area of purple basil was 26% to 44% lower with the inclusion of G wavelengths. Light quality treatments showed no effects on the SLA of basil, regardless of cultivar. No correlations between plant height and BP, RP, or R:B ratios were observed in green or purple basil. The leaf area of green basil was positively correlated with R:B ratios, with a coefficient of 0.9854, whereas the leaf area of purple basil was positively correlated with RP, with a coefficient of 0.9504.

The shoot FW and DW of green basil were both positively correlated with R:B ratios, with coefficients of 0.9929 and 0.9518, respectively. The shoot FW and DW of purple basil were both positively correlated with RP, with coefficients of 0.9587 and 0.8977, respectively. No correlations were observed between plant yield with BP. Among the three R&B light combinations, shoot FW and DW of green basil were both lowest under treatments R₅₁B₄₉ and R₇₆B₂₄; however, for purple basil, they were both lowest under R₅₁B₄₉ (Fig. 5A and B). The inclusion of G wavelengths decreased shoot FW and DW of purple basil by 28% to 35% and 27% to 41%, respectively, compared with R&B light combinations with similar BP, whereas it decreased the shoot FW of green basil by 15% under 12% BP (Fig. 5A and B).

Different light quality treatments showed no effects on the anthocyanin concentration of green or purple basil (Table 1). Among the three R&B light combinations, the flavonoids concentration in and antioxidant capacity of green basil were both highest under R₇₆B₂₄ and decreased under 12% or 49% BP, whereas the phenolics concentration was unaffected by BP. Among the three R&B light combinations, the phenolics concentration in purple basil increased with increasing BP, whereas the flavonoids concentration in and antioxidant capacity were highest under R₇₆B₂₄ and R₅₁B₄₉ and lowest under R₈₈B₁₂ (Table 1). Under 12% BP, the phenolics concentration in green basil decreased with the inclusion of G wavelengths, whereas the flavonoids concentration in and antioxidant capacity were unaffected. In contrast, under 12% BP, the phenolics concentration in purple basil was unaffected by the inclusion of G wavelengths, whereas the flavonoids concentration in and antioxidant capacity decreased. Under 24% BP, the phenolics concentration and flavonoids concentration in and antioxidant capacity of green and purple basil all decreased with the inclusion of G wavelengths. No correlations between phytochemical parameters and BP, RP, or R:B ratios were observed in green or purple basil.

The total amounts of anthocyanin, phenolics, and flavonoids in and antioxidant capacity per plant were calculated by multiplying the concentrations of each parameter with leaf FW per plant. Among the three R&B light combinations, the total amounts of anthocyanin in both cultivars were highest under R₈₈B₁₂ and R₇₆B₂₄ and the same as the total amounts of phenolics in green basil, whereas the total amount of flavonoids in and antioxidant capacity in both cultivars and the total amount of phenolics in purple basil were all highest under R₇₆B₂₄ (Table 2). The total amount of anthocyanin in green basil under both BP and the total amount of flavonoids in green basil under 12% BP were unaffected by inclusion of G wavelengths, whereas other total amounts of phytochemicals per plant (i.e., anthocyanin, phenolics, flavonoids, and antioxidant capacity) decreased with inclusion of G wavelengths, regardless of BP (Table 2).

Morphological and physiological responses in mustard to different RP, BP, and GP

Among the different light quality treatments, no difference was observed in the P_n or SPAD of green mustard and red mustard (Figs. 2 and 3).

The plant height and leaf area of green mustard were both negatively correlated with BP, with coefficients of -0.8958 and -0.8844, respectively, whereas the leaf area of green mustard was positively correlated with R:B ratios, with a coefficient of 0.9239. However, no correlations between growth parameters and BP, RP, or R:B ratios were observed in red mustard. The plant height and leaf area of green mustard decreased by 15% and 22%, respectively, when BP increased from 12% to 49% (Fig. 4C). The SLA of green mustard was lowest under 49% BP, whereas the inclusion of G wavelength decreased its SLA under 24% BP. The inclusion of G wavelengths increased plant height in green mustard under 12% BP but decreased it under 24% BP. Light quality showed no influence on the plant height or SLA of red mustard, whereas the inclusion of G wavelengths decreased the leaf area under 24% BP (Fig. 4D).

Among the three R&B light combinations, the shoot FW of green mustard was highest under R₈₈B₁₂, but it showed no difference between R₇₆B₂₄ and R₅₁B₄₉ (Fig. 5C). The shoot FW

Fig. 4. Plant height and leaf area of (A) green basil ‘Improved Genovese Compact’, (B) purple basil ‘Red Rubin’, (C) green mustard ‘Amara’, (D) red mustard ‘Red Giant’, (E) green kale ‘Siberian’, and (F) red kale ‘Scarlet’ grown under three combined red (R) and blue (B) light combinations, R₈₈B₁₂ (the proportions of R and B wavelengths were 88% and 12%, respectively), R₇₆B₂₄ (the proportions of R and B wavelengths were 76% and 24%, respectively), and R₅₁B₄₉ (the proportions of R and B wavelengths were 51% and 49%, respectively) and two white light combinations, R₄₄B₁₂G₄₄ [white light-emitting diode with R, B, and green (G) wavelength proportions of 44%, 12%, and 44%, respectively] and R₃₅B₂₄G₄₁ (white fluorescent light with R, B, and G wavelength proportions of 35%, 24%, and 41%, respectively). Means followed by different lowercase and uppercase letters indicate a significant difference for plant height and leaf area, respectively, according to one-way analysis of variance ($P \leq 0.05$).

of red mustard was unaffected by BP and RP (Fig. 5D), whereas the shoot FW of green mustard was positively correlated with R:B ratios, with a coefficient of 0.9100. The shoot FW of green mustard tended to decrease with the inclusion of G wavelengths, but the difference was not significant. The shoot DW of green mustard was unaffected by light quality treatments. The shoot FW and DW of red mustard were 32% and 29% lower with the inclusion of G wavelengths under 24% BP, respectively. No correlations between plant yield and BP were observed in mustard.

Among the three R&B light combinations, the phenolics concentration in green mustard increased with increasing BP, whereas its other phytochemical concentrations were unaffected by BP (Table 1). In red mustard, the anthocyanin concentration was highest under R₈₈B₁₂ and R₅₁B₄₉ and lowest

under R₇₆B₂₄. The other phytochemical concentrations in red mustard were unaffected by BP (Table 1). Under 12% BP, the phenolics concentration in green mustard decreased with the inclusion of G wavelengths, whereas it showed no effects on the other phytochemical concentrations, regardless of cultivar or BP (Table 1). The anthocyanin concentration in green mustard was negatively correlated with RP, with a coefficient of -0.9569 , and its phenolics concentration was negatively correlated with R:B ratios, with a coefficient of -0.8963 . No correlations between phytochemical parameters and BP, RP, or R:B ratio were observed in red mustard.

Among the three R&B light combinations, the total amounts of anthocyanin and flavonoids in green mustard were both lowest under 49% BP, whereas other total amounts of phytochemicals were unaffected by BP, regardless of cultivar (Table

Fig. 5. Shoot fresh weight and dry weight of (A) green basil ‘Improved Genovese Compact’, (B) purple basil ‘Red Rubin’, (C) green mustard ‘Amara’, (D) red mustard ‘Red Giant’, (E) green kale ‘Siberian’, and (F) red kale ‘Scarlet’ grown under three combined red (R) and blue (B) light combinations, R₈₈B₁₂ (the proportions of R and B wavelengths were 88% and 12%, respectively), R₇₆B₂₄ (the proportions of R and B wavelengths were 76% and 24%, respectively), and R₅₁B₄₉ (the proportions of R and B wavelengths were 51% and 49%, respectively), and two white light combinations, R₄₄B₁₂G₄₄ [white light-emitting diode with R, B, and green (G) wavelength proportions of 44%, 12%, and 44%, respectively] and R₃₅B₂₄G₄₁ (white fluorescent light with R, B, and G wavelength proportions of 35%, 24%, and 41%, respectively). Means followed by different lowercase and uppercase letters indicate a significant difference for shoot fresh weight and dry weight, respectively, according to one-way analysis of variance ($P \leq 0.05$).

2). Under 12% BP, the total amounts of anthocyanin and phenolics in green mustard increased with the inclusion of G wavelengths, whereas other total amounts of phytochemicals in green mustard were unaffected, regardless of BP. In red mustard, all total amounts of phytochemicals decreased with the inclusion of G wavelengths, regardless of BP, except with the total amount of flavonoids under 12% BP (Table 2).

Morphological and physiological responses in kale to different RP, BP, and GP

In green kale, comparative P_n was highest under treatments R₅₁B₄₉ and R₇₆B₂₄ (Fig. 2), which was positively correlated with BP,

with a coefficient of 0.9279, and showed no relationship with RP or R:B ratios. The SPAD reading of green kale was unaffected by light quality treatments (Fig. 3). In red kale, comparative P_n was not influenced by BP among three R&B light combinations, whereas the inclusion of G wavelengths decreased comparative P_n under 24% BP (Fig. 2). Increasing BP from 12% to 24% increased the SPAD of red kale among three R&B light combinations. Under 12% BP, the inclusion of G wavelengths increased the SPAD of red kale by 15%; however, under 24% BP, the inclusion of G wavelengths showed no effects (Fig. 3).

The plant height and leaf area of green and red kale both decreased with increasing BP among the three R&B light

Table 1. Concentrations of anthocyanin, phenolics, and flavonoids and antioxidant capacity of green basil ‘Improved Genovese Compact’, purple basil ‘Red Rubin’, green mustard ‘Amara’, red mustard ‘Red Giant’, green kale ‘Siberian’, and red kale ‘Scarlet’ grown under five light quality treatments, which include three combined red (R) and blue (B) light combinations, R₈₈B₁₂ (the proportion of R and B wavelength was 88% and 12%, respectively), R₇₆B₂₄ (the proportion of R and B wavelength was 76% and 24%, respectively), and R₅₁B₄₉ (the proportion of R and B wavelength was 51% and 49%, respectively) and two white light, R₄₄B₁₂G₄₄ [white light emitting-diode with R, B, and green (G) wavelength proportion of 44%, 12%, and 44%, respectively] and R₃₅B₂₄G₄₁ (white fluorescent light with R, B, and G wavelength proportion of 35%, 24%, and 41%, respectively).

Cultivar	Treatment	Anthocyanin concn (mg/100 g)	Phenolics concn (mg·g ⁻¹)	Flavonoids concn (mg·g ⁻¹)	Antioxidant capacity (mg/100 g)
Green basil	R ₈₈ B ₁₂	2.54 a ^z	1.28 a	0.49 bc	295 b
	R ₄₄ B ₁₂ G ₄₄	2.77 a	1.04 b	0.48 bc	290 bc
	R ₇₆ B ₂₄	2.77 a	1.37 a	0.84 a	389 a
	R ₃₅ B ₂₄ G ₄₁	2.95 a	1.01 b	0.40 c	249 c
	R ₅₁ B ₄₉	2.75 a	1.30 a	0.53 b	251 bc
Purple basil	R ₈₈ B ₁₂	13.5 a	2.81 c	2.43 b	1570 b
	R ₄₄ B ₁₂ G ₄₄	14.1 a	2.67 c	1.92 c	1394 c
	R ₇₆ B ₂₄	13.8 a	3.21 b	2.70 a	1755 a
	R ₃₅ B ₂₄ G ₄₁	13.9 a	2.62 c	1.87 c	1229 d
	R ₅₁ B ₄₉	14.1 a	3.47 a	2.70 a	1873 a
Green mustard	R ₈₈ B ₁₂	7.22 a	0.98 c	0.87 a	176 a
	R ₄₄ B ₁₂ G ₄₄	7.90 a	1.12 b	0.96 a	151 a
	R ₇₆ B ₂₄	7.53 a	1.13 b	0.96 a	186 a
	R ₃₅ B ₂₄ G ₄₁	7.86 a	1.12 b	0.95 a	172 a
	R ₅₁ B ₄₉	7.70 a	1.25 a	0.90 a	198 a
Red mustard	R ₈₈ B ₁₂	9.93 ab	1.22 a	0.86 a	221 ab
	R ₄₄ B ₁₂ G ₄₄	9.57 b	1.09 a	0.85 a	146 b
	R ₇₆ B ₂₄	9.47 b	1.21 a	0.87 a	213 ab
	R ₃₅ B ₂₄ G ₄₁	9.90 ab	1.27 a	0.85 a	232 a
	R ₅₁ B ₄₉	10.27 a	1.26 a	0.90 a	220 a
Green kale	R ₈₈ B ₁₂	6.74 b	0.75 c	0.97 a	141 bc
	R ₄₄ B ₁₂ G ₄₄	7.51 a	0.79 bc	1.01 a	112 c
	R ₇₆ B ₂₄	7.15 ab	0.81 bc	0.97 a	121 bc
	R ₃₅ B ₂₄ G ₄₁	7.61 a	1.11 a	1.07 a	178 a
	R ₅₁ B ₄₉	7.44 a	0.98 ab	1.04 a	151 ab
Red kale	R ₈₈ B ₁₂	9.73 a	1.40 bc	1.22 a	279 a
	R ₄₄ B ₁₂ G ₄₄	9.51 a	1.22 c	1.17 a	210 b
	R ₇₆ B ₂₄	9.61 a	1.47 ab	1.26 a	294 a
	R ₃₅ B ₂₄ G ₄₁	10.16 a	1.65 a	1.13 a	314 a
	R ₅₁ B ₄₉	9.74 a	1.64 a	1.22 a	334 a

^zMeans followed by the same lowercase letters are not significantly different according to one-way analysis of variance ($P \leq 0.05$).

combinations (Fig. 4E and F). The leaf area of red kale was negatively correlated with BP, with a coefficient of -0.8831 (Fig. 4F). The SLA of green kale and red kale were unaffected by light quality treatments. The inclusion of G wavelengths decreased plant height under 24% BP, regardless of cultivar, whereas it showed no effects under 12% BP (Fig. 4E and F). The inclusion of G wavelengths showed no effects on leaf area, regardless of cultivar or BP.

Among the three R&B light combinations, the shoot FW of green kale and red kale decreased by 18% and 24%, respectively, when BP increased from 12% to 49% (Fig. 5E and F). No correlations between plant yield and BP, RP, or R:B ratios were observed in green kale or red kale. Shoot DW of green kale and red kale were unaffected by BP. Under 24% BP, the inclusion of G wavelengths decreased the shoot FW by 21% to 28%, regardless of cultivar, but it showed no effects under 12% BP.

Among the three R&B light combinations, the anthocyanin concentration and phenolics concentration in and antioxidant capacity of green kale were highest under R₇₆B₂₄ and R₅₁B₄₉, whereas the flavonoids concentration was unaffected by light quality treatments (Table 1). A similar trend was observed for

the phenolics concentration in red kale, but its anthocyanin concentration, flavonoids concentration, and antioxidant capacity were unaffected by changing BP (Table 1). Under 12% BP, the inclusion of G wavelengths increased the anthocyanin concentration in green kale and decreased the antioxidant capacity of red kale, but it showed no effects on the other phytochemical concentrations. Under 24% BP, the inclusion of G wavelengths increased the phenolics concentration in and antioxidant capacity of green basil, but it showed no effects on the other phytochemical concentrations, regardless of cultivar. The anthocyanin concentration in green kale was negatively correlated with RP and R:B ratios, with coefficients of -0.9689 and -0.8948 , respectively, whereas its flavonoids concentration was negatively correlated with RP, with a coefficient of -0.8957 . No correlations between phytochemical parameters and BP, RP, or R:B ratio were observed in red kale.

Among the three R&B light combinations, increases of BP showed no effects on the total amounts of phenolics and flavonoids in and the antioxidant capacity of green kale, but its total amount of anthocyanin was lowest under treatment R₅₁B₄₉ (Table 2). In red kale, the total amount of anthocyanin was highest under

Table 2. Total amounts of anthocyanin, phenolics, and flavonoids and antioxidant capacity per plant of green basil ‘Improved Genovese Compact’, purple basil ‘Red Rubin’, green mustard ‘Amara’, red mustard ‘Red Giant’, green kale ‘Siberian’, and red kale ‘Scarlet’ grown under five light quality treatments, which include three combined red (R) and blue (B) light combinations, R₈₈B₁₂ (the proportion of R and B wavelength was 88% and 12%, respectively), R₇₆B₂₄ (the proportion of R and B wavelength was 76% and 24%, respectively), and R₅₁B₄₉ (the proportion of R and B wavelength was 51% and 49%, respectively) and two white light, R₄₄B₁₂G₄₄ [white light emitting-diode with R, B, and green (G) wavelength proportion of 44%, 12%, and 44%, respectively] and R₃₅B₂₄G₄₁ (white fluorescent light with R, B, and G wavelength proportion of 35%, 24%, and 41%, respectively).

Cultivar	Treatment	Total amount of phytochemicals (mg/plant)			
		Anthocyanin	Phenolics	Flavonoids	Antioxidant capacity
Green basil	R ₈₈ B ₁₂	0.51 a ^z	25 a	10 b	59 b
	R ₄₄ B ₁₂ G ₄₄	0.46 ab	18 c	8 bc	49 c
	R ₇₆ B ₂₄	0.49 ab	24 ab	15 a	68 a
	R ₃₅ B ₂₄ G ₄₁	0.48 ab	17 c	7 c	41 d
	R ₅₁ B ₄₉	0.44 b	21 b	9 b	40 d
Purple basil	R ₈₈ B ₁₂	2.01 a	42 b	36 b	234 b
	R ₄₄ B ₁₂ G ₄₄	1.39 c	26 c	19 d	138 c
	R ₇₆ B ₂₄	2.01 a	47 a	39 a	255 a
	R ₃₅ B ₂₄ G ₄₁	1.46 c	27 c	20 d	129 c
	R ₅₁ B ₄₉	1.71 b	42 b	33 c	227 b
Green mustard	R ₈₈ B ₁₂	3.14 b	43 b	38 ab	76 a
	R ₄₄ B ₁₂ G ₄₄	3.42 a	49 a	41 a	65 a
	R ₇₆ B ₂₄	2.96 bc	45 ab	38 ab	74 a
	R ₃₅ B ₂₄ G ₄₁	2.80 cd	40 b	34 bc	61 a
	R ₅₁ B ₄₉	2.67 d	42 b	31 c	69 a
Red mustard	R ₈₈ B ₁₂	4.70 a	58 a	41 ab	103 a
	R ₄₄ B ₁₂ G ₄₄	4.16 b	47 b	37 b	63 b
	R ₇₆ B ₂₄	4.85 a	62 a	42 a	101 a
	R ₃₅ B ₂₄ G ₄₁	3.32 c	43 b	29 c	78 b
	R ₅₁ B ₄₉	4.76 a	59 a	42 a	102 a
Green kale	R ₈₈ B ₁₂	3.33 b	37 a	48 ab	70 a
	R ₄₄ B ₁₂ G ₄₄	3.84 a	40 a	52 a	57 a
	R ₇₆ B ₂₄	3.42 b	39 a	46 abc	58 a
	R ₃₅ B ₂₄ G ₄₁	2.89 c	42 a	41 c	67 a
	R ₅₁ B ₄₉	3.01 c	40 a	42 bc	61 a
Red kale	R ₈₈ B ₁₂	3.48 a	50 a	44 a	100 a
	R ₄₄ B ₁₂ G ₄₄	3.06 c	39 b	38 b	68 c
	R ₇₆ B ₂₄	3.26 b	50 a	43 a	100 a
	R ₃₅ B ₂₄ G ₄₁	2.47 e	40 b	27 d	76 bc
	R ₅₁ B ₄₉	2.65 d	45 ab	33 c	91 ab

^zMeans followed by the same lowercase letters are not significantly different according to one-way analysis of variance ($P \leq 0.05$).

R₈₈B₁₂, followed by R₇₆B₂₄, and lowest under R₅₁B₄₉, whereas the total amount of flavonoids was highest under R₈₈B₁₂ and R₇₆B₂₄ and lowest under R₅₁B₄₉. The total amount of phenolics in and antioxidant capacity of red kale were unaffected by BP. The inclusion of G wavelengths increased the total amount of anthocyanin in green kale under 12% BP, whereas it decreased its total amount of anthocyanin under 24% BP, but it showed no effects on the other total amounts of phytochemicals in green kale, regardless of BP. In red kale, the inclusion of G wavelengths decreased all total amounts of phytochemicals, regardless of BP (Table 2).

Discussion

Photosynthesis and chlorophyll content as influenced by RP, BP, and GP

Although responses of plant photosynthesis to B wavelengths are species-specific and cultivar-specific, many studies

indicated that supplementing R with B wavelengths increased plant photosynthetic performance by enriching chlorophyll content, increasing stomatal density, and improving enzyme activity (Bugbee, 2016; Fan et al., 2013; Li, 2010). The chlorophyll concentration, stomatal conductance, and P_n in cucumber were positively correlated with BP ranging from 0% to 80% under R&B light combination (Hernandez et al., 2016; Hogewoning et al., 2010). In this study, we measured the effects of different waveband proportions on plant photosynthetic capacity, and the comparative P_n of purple basil and green kale were positively correlated with BP. This was supported by studies by Kinoshita et al. (2001) and Zeiger et al. (2002), in which the B wavelength alone or R&B light combination increased stomatal density and aperture, which may increase plants' capacity for CO₂ uptake and ultimately increase photosynthesis. Furthermore, activities of photosynthetic enzymes (e.g., Rubisco) and biosynthetic intermediates of chlorophylls (e.g., 5-aminolevulinic acid, protoporphyrin IX, Mg-protoporphyrin IX, and protochlorophyllide) increased under enriched B wavelengths (Bondada and Syvertsen, 2003; Fan et al., 2013; Li, 2010). Increasing B wavelengths can stimulate high irradiance leaf characteristics even under constant PPFD, performing as improved P_n with increasing BP under constant PPFD (Hogewoning et al., 2010). However, plant responses to B wavelength are different at whole-plant canopy and single-leaf scales

(Craver et al., 2020). Overall plant growth under high BP is inhibited due to B wavelength-inhibited extension growth, whereas single-leaf photosynthesis is promoted (Bugbee, 2016; Craver et al., 2020). Results of the present study support these findings, and inhibition of overall plant growth by B wavelengths is presented later.

In this study, the inclusion of G wavelengths decreased the comparative P_n in red kale, and a similar trend was observed in green kale and mustard (Fig. 2). It was postulated that G wavelength deactivation of B wavelength-activated cryptochrome removes the signal for suppression of abscisic acid production in guard cells, resulting in a decreased stomatal aperture and stomatal opening, subsequently decreasing P_n (Meng et al., 2019). In particular, the flavin chromophore of cryptochrome is driven to a biologically active semi-reduced form by B wavelengths, which is shifted to a deactivated reduced state by the absorption of G wavelengths (Banerjee

et al., 2007; Bouly et al., 2007). Similarly, Kang et al. (2016) reported that the inclusion of 10% GP decreased P_n and chlorophyll concentrations in 'Green Skirt' lettuce compared with plants grown under R&B light combinations (BP ranging from 10% to 30%).

Plant growth and yield as influenced by RP, BP, and GP

Previous studies have indicated that different light wavelengths can regulate not only plant photosynthesis but also plant growth and development. Particularly, R wavelengths stimulate plant extension growth (i.e., stem elongation and leaf expansion) via phytochrome-mediated pathways, whereas B wavelengths inhibit plant extension growth via cryptochrome-mediated and phototropin-mediated pathways (Bugbee, 2016). In this study, the plant yield of all tested species decreased with increasing BP, except for red mustard, which showed a trend similar to that of plant height and leaf area (Figs. 4 and 5) and opposite that of comparative P_n (Fig. 2). This indicated that plant biomass accumulation is predominantly related to plant extension growth (i.e., plant height, leaf area) rather than plant photosynthetic capacity. Plant extension growth and P_n are two key elements determining plant photosynthetic capacity; the former determines the plant light interception capacity and the latter indicates the instantaneous gas exchange rate per unit of leaf area. Our results were consistent with those of Hernandez et al. (2016), who reported that plant height, leaf area, and shoot FW and DW of 'Cumlaude' cucumber decreased with increasing BP from 10% to 75%, whereas P_n , stomatal conductance, and chlorophyll concentrations showed an opposite trend. Similarly, leaf length and width of 'Green Skirt' lettuce decreased with increasing BP from 0% to 30% (Kang et al., 2016).

Shade avoidance responses such as the promotion of hypocotyl elongation, stem elongation, and leaf expansion are evoked by G wavelengths in a range of plant species and have two categories: cryptochrome-dependent pathways and cryptochrome-independent pathways (Folta, 2004; Wang and Folta, 2013; Zhang et al., 2011). However, the inclusion of G wavelengths at high proportions reduces the photon flux of R and/or B wavelengths under constant PPFD, which may result in negative effects on plant growth (Folta and Maruhnich, 2007). For example, leaf area, leaf thickness, and plant yield of 'Waldmann's Green' lettuce increased under 24% GP ($R_{61}B_{15}G_{24}$) compared with plants grown under the R&B light combination ($R_{84}B_{16}$), whereas treatment with 51% GP ($R_{30}B_{19}G_{51}$) showed no effects on leaf area or yield, and treatments with 86% GP ($R_4B_{10}G_{86}$) decreased the leaf area and yield (Kim et al., 2004). In this study, due to the high GP (41% and 44%), the inclusion of G wavelengths reduced the leaf area and subsequently reduced the shoot FW and DW.

A novel finding of this study is that the effects of the inclusion of G wavelengths on stem elongation differed under different RP and/or BP or R:B ratios. In particular, under low BP of 12%, the inclusion of G wavelengths increased the plant height of green basil and green mustard; however, under high BP of 24%, the inclusion of G wavelengths decreased plant height in most tested species (Fig. 4). It was postulated that under low BP of 12%, which has a relatively low B photon flux and high R:B ratio (88:12 and 44:12), G wavelengths reverse the B wavelength inhibition responses and dominate in stem elongation, resulting in higher plant height. Under high BP of 24%, a relatively high B photon flux and low R:B ratio (76:24

and 35:24) together may suppress stem elongation and override the weak control of expansion growth by G wavelengths, resulting in shorter plant height. Wang and Folta (2013) also reported that the hypocotyl length of arabidopsis (*Arabidopsis thaliana*) increased with additional G wavelengths to a low R and B photon flux ($<10 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and showed no effects at a high R and B photon flux ($\geq 10 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). This suggests that plant responses to G wavelengths not only depend on GP or G photon flux in total PPFD but also are influenced by the BP or R:B ratio.

Secondary metabolites accumulation as influenced by RP, BP, and GP

Although both R and B wavelengths are involved in the synthesis of secondary metabolites, B wavelengths enriched the concentrations of phytochemicals compared with R wavelengths in most cases (Lobiuc et al., 2017; Qian et al., 2016). For example, the anthocyanin concentration in lettuce, sage, and kale increased under higher BP with constant PPFD, as did the phenolics concentration in purple basil (Hosseini et al., 2018; Li, 2010; Meng et al., 2004; Qian et al., 2016). Similarly, the antioxidant capacity of lettuce and kale both increased under enriched B wavelengths (Qian et al., 2016; Son and Oh, 2013). In this study, increases in BP enriched the concentration of anthocyanin in green kale, the concentration of phenolics in all tested species except green basil and red mustard, and the concentration of flavonoids in and antioxidant capacity of purple basil (Table 1). Induced synthesis of secondary metabolites by B wavelengths is supported by Li (2010) and Meng et al. (2004), who reported that the 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 B wavelengths. In contrast, increases in BP decreased the total amount of phytochemicals per plant, which was consistent with the trend of shoot FW (Fig. 5; Table 2). Therefore, two mechanisms of increased phytochemical concentrations under higher BP were postulated: 1) higher BP induced the expression of key enzymes in the phenylpropanoid pathway, such as PAL, CHS, and DFR, which induced the synthesis of anthocyanin, phenolics, and flavonoids (Li, 2010; Meng et al., 2004); and 2) reduced phytochemical concentrations under higher RP could be attributed to the dilution of phytochemicals at higher plant biomass. More research is needed to identify the mechanism of B wavelength-enriched phytochemical concentrations.

Interactions between G and R&B light combinations are difficult to predict but are known to be mediated by photosensory pathways because photoreceptors such as phytochromes, phototropins, and cryptochromes also absorb G wavelengths (Folta and Maruhnich, 2007; Wang and Folta, 2013; Zhang and Folta, 2012). Specifically, plant responses to G wavelengths share a general tendency to counteract the B wavelength-induced anthocyanin accumulation in a range of plant species such as arabidopsis and lettuce (Talbot et al., 2006; Zhang et al., 2011; Zhang and Folta, 2012). Pennisi et al. (2019) reported that G wavelengths ($R_{29}B_{31}G_{40}$) significantly decreased the antioxidant capacity of and flavonoid concentration in basil compared with plants grown under R&B light combinations ($R_{62}B_{30}$). However, the inclusion of G wavelengths showed both positive and negative effects on phytochemical accumulation in this study (Tables 1 and 2). With similar BP, the decreased phytochemical concentration with the

inclusion of G wavelengths was postulated to be caused by the G wavelength reversal of B wavelength-induced phytochemical synthesis (Zhang and Folta, 2012). However, the concentration and total amount of anthocyanin in green kale both increased with the inclusion of G wavelengths under 12% BP, suggesting a novel pathway for anthocyanin synthesis induced by G wavelengths. One hypothesis is that under low BP of 12%, the inclusion of G wavelengths may increase the high-energy wavelengths intercepted by green kale compared with R&B light combinations with similar BP, which induced anthocyanin synthesis as a stress response.

Conclusion

The results of this study suggest that adjusting the suitable spectrum of light wavelengths may provide important tools for regulating plant photosynthesis, morphology, biomass accumulation, and phytochemical synthesis. Increases in BP improved plant photosynthesis and phytochemical concentrations but suppressed plant expansion growth, yield, and the total amount of phytochemicals per plant. Effects of the inclusion of G wavelengths on stem elongation differed under different RP and/or BP and R:B ratios, and relatively high GP negatively influenced plant photosynthesis, chlorophyll content, leaf area, and yield. The inclusion of G wavelengths showed both positive and negative effects on phytochemical accumulation in this study. More research is required to clarify the effects of G wavelengths on secondary metabolites synthesis.

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