The influence of LEDs with different blue peak emission wavelengths on the biomass, morphology, and nutrient content of kale cultivars

Eyosias L. Ashenafi [a], Marianne C. Nyman [a]*, Jake M. Holley [b], Neil S. Mattson [b]

[a] Department of Civil and Environmental Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180, USA

[b] School of Integrative Plant Science, Cornell University, Ithaca, NY 14583, USA

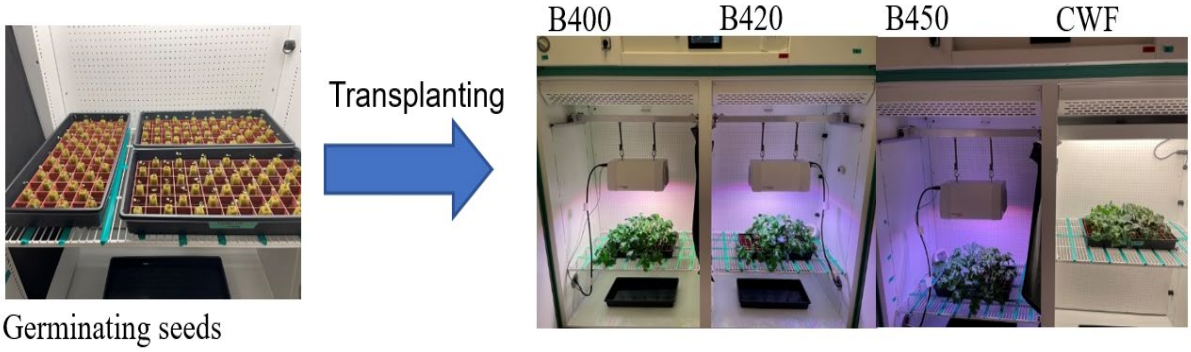
*Corresponding author: Dr. Marianne Nyman, 518-276-2268, Email: nymanm@rpi.edu

Abstract

Light-emitting diodes (LEDs) enhance plant production in vertical farms by regulating photosynthetic rate and phytochemistry. Specific light recipes can be formulated using LEDs for high output by fine-tuning spectral composition and irradiance. In this study, the growth, development, and nutritional quality of three kale cultivars ('Toscano', 'Redbor', and 'Winterbor') were examined under different blue peak emission wavelengths (λ_{peak}). Photosynthetic photon flux density (PPFD) was maintained at 200 ± 10 % µmol m^{-2} s^{-1} over a 16-hr photoperiod. The LED light treatments had blue λ_{peak} centered at 400, 420, and 450 nm wavelengths, all with spectral ratios of 20 % blue, 20 % green, 60 % red in the visible light region, and 15 % of total PPFD in the far-red region. The control light was cool-white fluorescent (CWF) light with blue λ_{peak} at 436 nm and a slightly higher amount of PPFD in the blue region (23%). The biomass yield and leaf physical characteristics were largely unaffected by the light treatments with different blue λ_{peak}. However, the concentration of carotenoids and chlorophylls in kale leaves was influenced by the type and amount of blue light during growth. Future research should investigate the effect of different blue light percentages in pre- and post-harvest LED treatments (continuous or pulsed) on high-value, nutritious crops.

Keywords: carotenoids, phytochemicals, blue light, controlled environment, light-emitting diode, kale

Graphical abstract



1. Introduction

Light emitting diodes (LEDs) can be designed with built-in dimmable (intensity control) and tunable (spectral control) functions to enhance crop productivity in indoor plant factories [1]. Recently, several research groups have been employing LED fixtures with independently controllable light channels and narrow spectral regions for identifying optimum light recipes for growing different crops [2-4]. Compared to High-Pressure Sodium or fluorescent light sources, LEDs have long lamp life (up to 50,000 hrs.), minimal infrared emission, and high electrical efficiency [5-7]. Over time, advancements in semiconductor technology will allow for the manufacture of LEDs with a significantly increased light output, while the production cost of LEDs is projected to plummet over time [8].

Previous research demonstrates that plants growing under monochromatic (100 %) blue or red light display abnormal growth and low yield in biomass and pigment content [9-10]. Hence, an investigation into photomorphogenesis and pigment biosynthesis under a combination of different light spectra is warranted. The most efficient and cost-effective way to maximize yield and quality of plant growth involves using light to interact with both photosynthesis and plant photoreceptors. One common strategy is to match the emission spectrum of the light source with the absorption spectra of photosynthetic pigments and photoreceptors in plant leaves for a high probability of photochemical reactions [11-12].

Light sources with high red: blue (R:B) ratio have been shown to promote plant growth and increase biomass accumulation [13-15]. On the contrary, a light spectrum with a low R:B ratio leads to increased production of important phytochemicals in

different plant species but at the cost of lower biomass yield [12,16-17]. A balance between promoting growth and increasing phytochemicals is necessary for optimum plant production.

Light absorption of blue and red light is above 90 %, while only 81 % of incident green light is absorbed by leaves [2]. This is due to the high absorption of blue and red light by the photosynthetic pigments – chlorophylls and carotenoids [18]. However, green light is necessary to increase photosynthetic productivity, particularly in lower canopy plants due to its deeper penetration than other light photons (high transmittance of green light by uppermost leaves, especially at high PPFDs) [2,19-20].

The interaction of blue light and blue-sensitive photoreceptors (cryptochromes, phototropins, and zeitlupes) controls aspects of plant physiology such as the production of secondary metabolites [12]. Known biological responses to high blue light treatment (40 % or more blue light) include lower biomass yield, compact growth, high stomatal conductance, and high phenolic content in comparison to light treatments lower in blue light [14,20–22]. Leaves with deep red or purple pigmentation (high anthocyanin content) are observed in plants grown under high blue light [14,23-24]. Blue light photons at different wavelengths have different relative quantum efficiency (RQE) for photosynthesis. RQE of monochromatic blue light at 400 nm wavelength is 0.66, while the RQE at 420, 436, and 450 nm is 0.75 [25]. It is important to note there are synergistic effects that take place when blue light is combined with other types of light [26].

Ying and co-workers [27] observed no effect of increasing blue light from 5 to 30 % in blue-red combination LED treatment on the concentration of photosynthetic

pigments and the concentration of harmful nitrate content in ‘Red Russian’ kale samples. However, a higher concentration of anthocyanins and total phenolic content (TPC) was observed when the ratio of blue light increased. In an experiment with two lettuce cultivars, the highest concentration of chlorophylls, anthocyanins, and total phenolics was reported in plants grown with “blue-rich” light (50 and 80 % blue), in comparison to monochromatic red/blue and simulated sunlight conditions [23]. A linear decrease in cotyledon area and hypocotyl length in kale and mustard seedlings was observed with an increase in the blue light fraction from 5 to 30 % [27]. However, no significant differences in fresh and dry weight were found in the seedlings of kale, arugula, and mustard plants grown under different combinations of blue and red light [27].

Kale, (*Brassica oleracea* L. var. *acephala*) a cold-tolerant, cruciferous vegetable, contains a diverse class of health-promoting, bioactive compounds. Previously, high concentrations of carotenoids (lutein and *β*-carotene), phenolics (anthocyanins), and glucosinolates have been measured in the leaves of kale cultivars [26-30]. These compounds provide good antioxidant properties, pro-vitamin A activity, and promote good cardiovascular and ocular health in humans [31]. Carotenoids in plant cells play important role in photoprotection by quenching radical species with high oxidizing potential during high light overexcitation [32].

Overall, past photobiological research primarily focused on understanding the effect of different light combinations on plant species but not the impact of peak emission wavelengths. In this study, the growth and development of three kale cultivars (‘Toscano’, ‘Redbor’, and ‘Winterbor’) at different λ_{peak} (wavelength of peak emission) of

125 blue light were investigated. Physical attributes (fresh weight, total leaf area, etc.) and
126 nutritional quality (chlorophyll, carotenoids, and total phenolics) of leaf samples were
127 quantified. We hypothesized that light treatment with a low peak wavelength for blue
128 light will promote a higher accumulation of bioactive compounds.
129

2. MATERIALS AND METHODS

2.1 Planting Materials and Growing Conditions

Seeds for the kale cultivars, 'Toscano', 'Redbor', and 'Winterbor', were purchased from Johnny's Selected Seeds (Winslow, ME). The seeds were sown on soaked Rockwool cubes (Grodan®, Milton, ON) inside Terra Cotta plant trays. Upon germination, kale seeds were transplanted to different light treatments and supplied with a nutrient solution prepared from 5-12-26 N-P-K Jack's hydroponic and CAL-Trate LX formula (JR Peters Inc, Allentown, PA). Tap water was used to prepare the nutrient solution. The nutrient composition was 200 ppm N, 50 ppm P, 206 ppm K, 78.4 ppm S, 182.7 ppm Ca, 60.3 ppm Mg, 3.63 ppm Fe, 0.63 ppm B, 0.86 ppm Mn, 0.52 ppm Zn, 0.22 ppm Cu, and 0.17 ppm Mo as described by Ashenafi [33]. Fresh nutrient solution was added to trays regularly as needed.

The effect of light treatments with different blue λ_{peak} on the growth and development of kale cultivars was examined. The LED treatments were performed using Heliospectra DYNA RX-30 LED lamps (Göteborg, Sweden). The λ_{peak} of the blue light channels listed by the LED manufacturer was 400 nm (B400), 420 nm (B420), and 450 nm (B450). Measured emission peaks of blue wavebands from spectroradiometer readings were 412 nm (for B400), 426 nm (for B420), and 457 nm (for B450). All LED treatments contain 20 % blue, 20 % green, 60 % red of PAR, and far-red (FR) light at 15 % of total PPFD.

The control light was cool-white fluorescent (CWF), which was supplied by Philips T5 CWF lamps (Eindhoven, Netherlands). Measured photon flux density in the

CWF light spectrum was 23 % blue (λ_{peak} = 436 nm), 44 % green, and 33 % red of the total PPFD and small FR light (4 % of PPFD). For all treatment groups, the light level was maintained at PPFD of 200 ± 10 % μmol m^{-2} s^{-1} at canopy height during the growth of kale. The spectral power distribution of the light sources was measured using a PS-300 spectroradiometer (Apogee Instruments, Logan, UT) and presented in **Fig. 1**.

Kale seeds were germinated in low CWF light for one week. Germinated plants were transplanted to growth chambers with the different light treatments (B400, B420, B450, and CWF) and grown inside A2000 growth chambers (Conviron, Winnipeg, MB). Plants were harvested and analyzed 30 days after seeding. Under the four light treatments, there was a total of three biological replicates for each cultivar. The indoor air temperature was set at 24 ºC during the 16-hr light period and 18 ºC during the 8-hr darkness. Relative humidity inside the chambers was maintained between 50 and 70 %. The planting area was assessed in a grid system and the light intensity in each grid was measured using a LI-190R quantum sensor (LI-COR Biosciences, Lincoln, NE). The measured intensity values at the center of the growing area were between 211.8 – 219.9 μmol m^{-2} s^{-1}. Plants were randomly selected for morphology and nutrient analysis to avoid potential differences based on spatial location within the chamber.

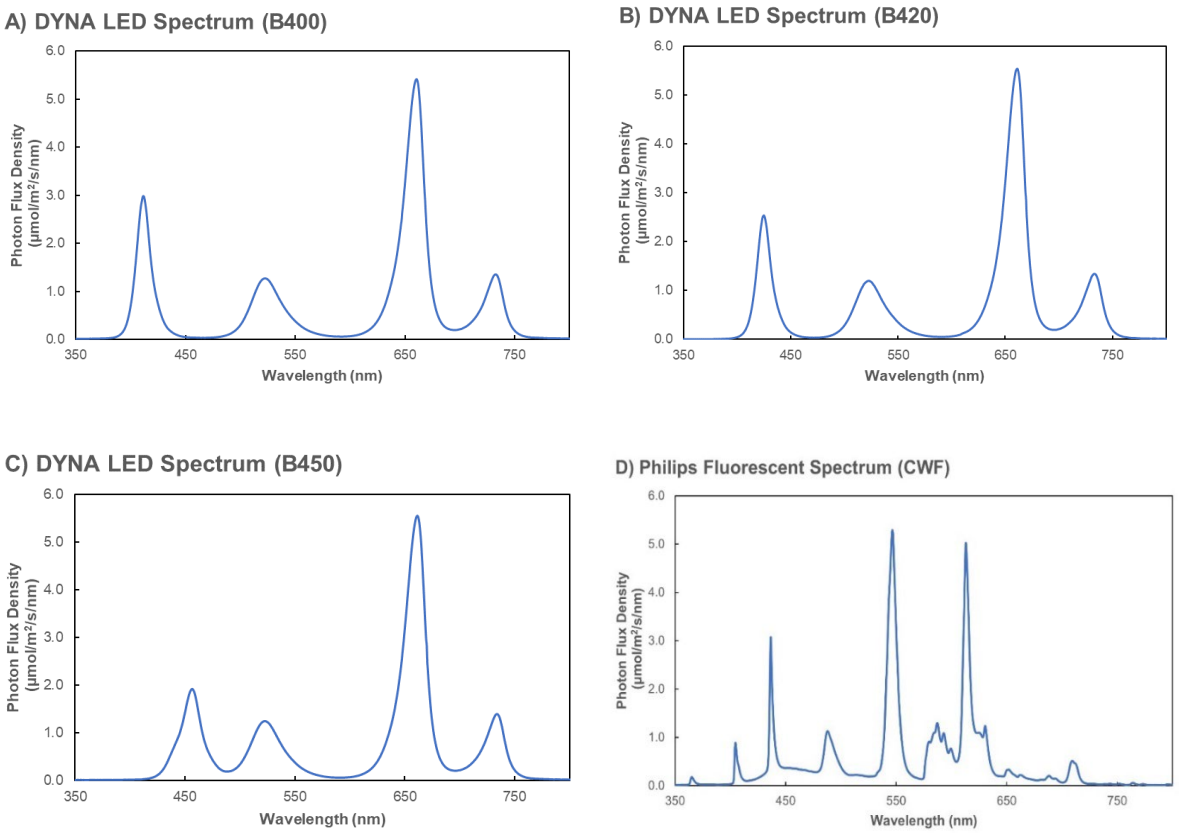


Fig. 1. Spectral power distribution of four light treatments. The first three LED treatments had different peaks for the blue emission waveband: 400 nm (A), 420 nm (B), and 450 nm (C). The control light was a cool white fluorescent lamp (D). **[Two column fitting]**

177 **2.2 Morphology measurements**

178 Five samples per planting cycle (a total of 15 plants) were harvested from each
179 treatment group for biomass and morphology measurements. Fresh weight (FW), dry
180 weight (DW), and total leaf area (TLA) of plant samples were measured. DW was found
181 by drying leaves inside the oven at 70 ºC until a constant weight was obtained. TLA was
182 measured using a CI-202 laser leaf area meter (CID-Bioscience, Camas, WA)**.** Moisture
183 content was determined based on the difference between FW and DW. Stem length and
184 diameter were measured using a transparent ruler and digital caliper, respectively.
185 Specific-leaf area was calculated using **Equation 1**, based on TLA and DW values.

$$\mathrm{SLA} = \frac{\mathrm{TLA}}{\mathrm{DW}} \qquad \textbf{Eqn. 1}$$

186 Where SLA is specific leaf area ($cm^2\ g^{-1}$); TLA is total leaf area (cm^2), and DW is the
187 total dry weight of leaf samples (g).

188 **2.3 Chlorophyll and carotenoid analysis**

189 At harvest time, four leaf samples from each light treatment were flash frozen,
190 placed in aluminum envelopes, and stored inside a - 80 ºC freezer until pigment
191 analysis. Frozen samples were homogenized with mortar and pestle prior to extraction.
192 Pigments from approximately 50 mg of plant matter (weight known) were extracted
193 using 1 mL of 80:20 (v/v) acetone: deionized (DI) water. Samples were vortexed and
194 centrifuged inside 1.5 mL plastic microcentrifuge tubes until only white pellets were
195 visible.

196 HPLC analysis was performed on a supernatant solution from the extracted leaf
197 samples for the determination of chlorophyll and carotenoid content. The analysis was
198 performed on a Prominence *i* LC-2030 3D liquid chromatograph with a PDA detector
199 (Shimadzu Scientific Instruments, Kyoto, Japan). Polymeric YMC C-30 Carotenoid
200 column (4.6 x 250 mm x 5 µm) was used as a stationary phase (YMC America,
201 Allentown, PA). The used mobile phase was isocratic 81:15:4 (v/v/v) methanol: methyl
202 *tert*- butyl ether: DI water solution. The flow rate was 1 mL min^{-1}.

203 High purity pigment standards were used for the analysis of pigments in extract
204 solutions. Lutein pigment standard (98.6 % purity) was obtained from ChromaDex
205 (Irvine, CA), while the standards for violaxanthin (≥ 95 %), chlorophyll *a* (≥ 85 %), and
206 chlorophyll *b* (≥ 90 %) were purchased from Sigma-Aldrich (St. Louis, MO). The peaks
207 of individual leaf pigments were identified using external standards under a run time of
208 36 minutes.

209

210

2.4 Total phenolic content analysis

Phenolic content was measured using a Folin-Ciocalteu reagent, according to a modified protocol from Ainsworth and Gillespie [34]. Pigments from approximately 20 mg (weight known) of homogenized leaf powder were extracted using a 95:5 (v/v) methanol: DI water solution. To each supernatant and standard solutions (200 µL volume), 10 % (v/v) Folin-Ciocalteu reagent (400 µL volume) and 0.7 mM sodium carbonate (1600 µL volume) were added. The reaction resulted in a dark-blue pigment complex. The mixture was incubated at room temperature for 2 hours. Absorbance at 765 nm wavelength was measured from each supernatant, blank, and gallic acid standards using quartz cuvettes and a UV-VIS spectrophotometer (UV-1800, Shimadzu Instruments, Kyoto, Japan). The TPC of kale samples was determined in gallic acid equivalents (GAE). Calibration standards of gallic acid were prepared in the 0.05 – 2.5 mM range in 95:5 (v/v) methanol: DI water. The coefficient of determination (r^2) value of gallic acid's calibration curve was 0.99. Folin-Ciocalteu Phenol TS solution was obtained from Spectrum Chemical Manufacturing (New Brunswick, NJ). Gallic acid standard (98 %) was purchased from Acros Organics (Fair Lawn, NJ).

2.5 Leaf gas exchange measurements

Water use efficiency (WUE) from each light treatment was measured using CIRAS-3 Portable Photosynthesis System (PP Systems, Amesbury, MA). Leaf gas exchange measurements were taken on the third fully expanded leaf under ambient light. The measurements were performed at each biological replicate (9 measurements per cultivar per treatment). The exchange of carbon dioxide (CO_2) and water (H_2O) molecules between leaves and gas cuvette was directly measured inside growth chambers, 1 hour after the photoperiod started and one or two days before harvest. Calculated parameters from direct measurements include net assimilation rate or A_n (μmol CO_2 m^{-2} s^{-1}), stomatal conductance or gs (mmol H_2O m^{-2} s^{-1}), and WUE (mmol CO_2 mol^{-1} H_2O). Fresh reference CO_2 cartridges were used to supply 400 ppm CO_2 inside leaf cuvettes during each day of measurement. The cuvette and analyzer flows were 300 and 100 mL min^{-1}, respectively.

2.6 Lamp efficiency measurement

The electrical consumption of LED lamps was measured using Kuman KW-47 electric meter (Shenzhen, China). Photosynthetic photon efficiency (PPE) of electric lamps can be calculated using **Equation 2.** The T5 CWF lamps were connected directly to the Conviron growth chambers for power, and hence a measurement of electrical consumption rate was not obtained. From previous measurements, PPE values of 0.84 – 0.95 μmol J^{-1} were reported in different fluorescent lamps [35].

$$\text{Photosynthetic Photon Efficiency} = \frac{\text{Number of PAR photons (μmol)}}{\text{Electrical energy input (J)}} \qquad \textbf{Eqn. 2}$$

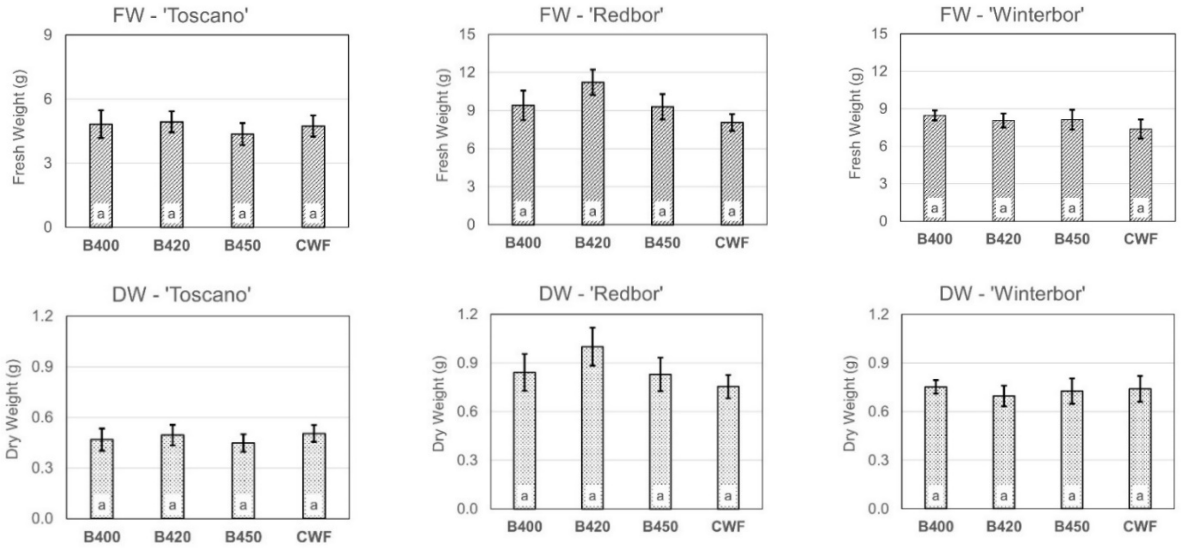
2.7 Statistical analysis

Mean of biomass, nutrient, and gas exchange measurements from different light treatments were compared using one-way ANOVA followed by Tukey's HSD at a 0.05 significance level. Different Tukey letters between different treatment groups indicate statistically significant differences with a > b > c. Pseudo-replicate measurements from HPLC and UV-VIS analysis were removed before statistical tests. Results are presented as mean ± standard error (SE) for different parameters. All statistical analysis was performed using R Studio Ver. 1.4.1103 (Boston, MA) and plotted using Microsoft Excel (Redmond, WA).

3. RESULTS AND DISCUSSION

3.1 Biomass analysis

At the end of the growth period, plant samples were harvested and the differences in physical characteristics between the light treatment groups were analyzed. In this study, no statistical differences were found in the FW and DW values between plants grown under the different LED and CWF treatments (see **Fig. 2**). This observation was apparent in all kale cultivars investigated. Plants in each treatment received the same total amount of light (DLI) during growth, which likely contributed to the insignificant differences in biomass measurements. Kurosaki [36] found similar findings with lettuce cultivars ‘Red Oak’ and ‘Rex’ grown under blue light at 412, 425, or 454 nm. In addition, Spalholz and co-workers [23] concluded that there was evident of gain in fresh mass for lettuce while exposing the lettuce to a variety of blue: red and sun simulated light treatments.



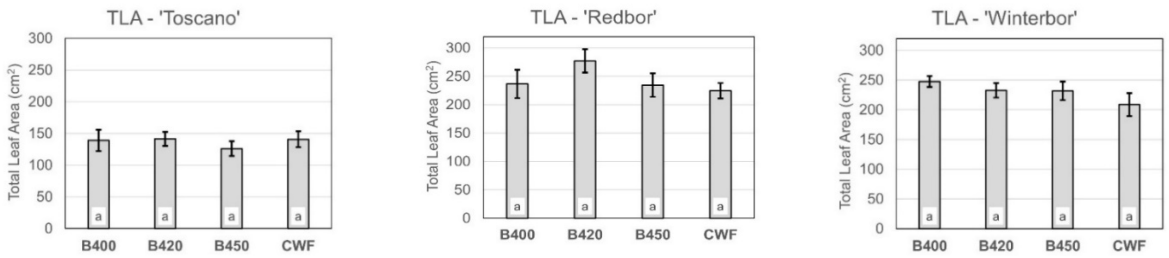


Fig. 2. Fresh weight (FW), dry weight (DW), and total leaf surface area (TLA) of three kale cultivars grown under the three LED and CWF (control) light treatments with different blue λ_{peak}. Error bars represent mean ± SE from three biological replicates. Sample size per cultivar: *n* = 15. Tukey's significance letters are indicated inside bar plots at the bottom. **[Two column fitting]**

Based on the electrical consumption of the LED spectra employed in this study, the PPE values (average of three measurements) were 1.86 µmol J^{-1} (B400), 2.01 µmol J^{-1} (B420), and 2.09 µmol J^{-1} (B450) over a unit area (m^2) at the specified intensity levels. The LEDs (regardless of the blue peak wavelength) had about twice the electrical efficacy as what has been reported in the literature for fluorescent fixtures [35]. Between the blue LED treatments, the B450 light was more efficient than the others. In other words, a higher amount of electrical energy was converted to PAR light in the B450 LED treatment; however, the photochemical action on selected kale plants was similar to B400, B420, and CWF light, based on the obtained yield data.

Dou and co-workers [13] found similar shoot FW, DW, and TLA for green basil plants grown under four LED treatments with different light spectra but identical PPFD (220 µmol m^{-2} s^{-1}). Similarly, Camejo and co-workers [37] observed non-significant differences in biomass yield in lettuce plants (cv. 'Batavia Lettony') grown under CWF and two LED light spectrums, all maintained at constant PPFD of 250 µmol m^{-2} s^{-1}. This phenomenon was also observed in 'Red Cos' lettuce grown under different combinations of blue, green, red, and UV light (all at constant irradiance) and harvested

295 at the seedling stage [38]. However, a decrease in final biomass has been found in
296 'Rouxai' lettuce when the blue light intensity was increased from 0 to 100 μmol m^{-2} s^{-1}
297 while maintaining the total PPFD constant [14].

298

3.2. Morphology analysis

Some differences were observed during the morphological analysis of kale cultivars from the different light treatments (see **Table 1**). Plants in the three LED groups had similar stem lengths, which were significantly longer than plants grown under the CWF light. CWF light spectrum contains a higher fraction of blue light (23 % Blue) and very low FR light (4 % of PPFD in FR) than the blue LED treatments (20 % Blue and 15 % of PPFD in FR) (see **Fig. 1**). Similarly, reduced stem elongation had been observed in lettuce, kale, and other leafy greens grown under light treatments with a high blue light percentages [16,20-21]. High FR light in the blue LED treatments can increase leaf expansion and internode elongation, while low FR light can elicit compact growth [3]. In addition, Vastakaite-Kairiene *et al.* [39] observed end-of-day blue light caused increased shoot elongation at all developmental growth stages of lettuce (*Lactuca sativa,* Lobjoits Green Cos) . However, Li and co-workers [40] found end-of-day blue light suppressed the growth of 'Red butter' lettuce, but enhanced the biomass yield in 'Green butter' lettuce.

Some significant differences were observed in the moisture content (%) of 'Toscano' and 'Winterbor' kale leaf samples (**Table 1**). Overall, 89 – 92 % moisture content was found in the three kale cultivars. No statistical differences were found in the SLA (inverse of leaf mass area) and stem diameter values between plants grown under the LED and CWF light. The calculated SLA values in this study were high (290 – 320 $cm^2\ g^{-1}$), which is characteristic of indoor-grown plants [37-38]. Slightly higher SLA values were found in 'Toscano' and 'Winterbor' kale grown in the blue LED treatments

321 when compared to the same cultivars grown under CWF light. For ‘Redbor’ kale, larger
322 but non-significant SLA values were found in CWF light treatments.
323

Table 1. Effect of blue peak wavelength on the morphology and moisture content of 'Toscano', 'Redbor', and 'Winterbor' kale. Values are reported as mean ± SE. Sample size n = 12.

A) 'Toscano'

Light treatment	SLA ($cm^2\ g^{-1}$)	Stem length (cm)	Stem diameter (mm)	Moisture content (%)
B400	320 ± 14.2a	7.9 ± 0.4a	3.1 ± 0.1a	90.6 ± 0.2a
B420	306 ± 14.5a	8.2 ± 0.4a	3.4 ± 0.1a	90.2 ± 0.3ab
B450	293 ± 11.4a	7.0 ± 0.4a	3.3 ± 0.2a	89.6 ± 0.4ab
CWF	289 ± 10.7a	4.3 ± 0.3b	3.5 ± 0.1a	89.2 ± 0.3b

B) 'Redbor'

Light treatment	SLA ($cm^2\ g^{-1}$)	Stem length (cm)	Stem diameter (mm)	Moisture content (%)
B400	301 ± 13.8a	7.6 ± 0.3a	4.3 ± 0.2a	91.3 ± 0.3a
B420	295 ± 14.1a	7.8 ± 0.3a	4.8 ± 0.2a	91.3 ± 0.3a
B450	307 ± 16.7a	6.7 ± 0.4a	4.5 ± 0.2a	91.3 ± 0.3a
CWF	314 ± 16.3a	4.3 ± 0.1b	4.6 ± 0.2a	90.7 ± 0.3a

C) 'Winterbor'

Light treatment	SLA ($cm^2\ g^{-1}$)	Stem length (cm)	Stem diameter (mm)	Moisture content (%)
B400	335 ± 12.7a	9.7 ± 0.4a	3.5 ± 0.1a	91.1 ± 0.2ab
B420	357 ± 19.3a	9.8 ± 0.3a	3.6 ± 0.1a	91.5 ± 0.3a
B450	337 ± 14.5a	9.1 ± 0.5a	3.5 ± 0.1a	91.1 ± 0.2ab
CWF	300 ± 15.9a	4.7 ± 0.3b	3.5 ± 0.1a	90.1 ± 0.4b

Tukey's significance letters with a > b at α = 0.05 significance level. Light treatments with the same significance letters are not statistically significant.

SLA = specific leaf area, B400 = Blue λ_{peak} at 400 nm, B420 = Blue λ_{peak} at 420 nm, B450 = Blue λ_{peak} at 450 nm, and CWF = Cool-white fluorescent.

3.3. Nutrient and Pigment analysis

HPLC analysis of leaf pigments was performed on harvested plants from the different light treatment groups. The concentration of lutein, violaxanthin, chlorophyll *a,* and chlorophyll *b* in leaves was measured. For all three kale cultivars, lutein content in the CWF light treatments (22.7 – 36.7 mg per 100 g of FW) was significantly higher than the lutein concentration found in the LED treatments (see **Fig. 3**). For instance, measured lutein concentration in 'Toscano' leaves from CWF treatment was 37.4 %, 32.4 %, and 28.3 % larger than LED treatments with blue λ_{peak} at 400, 420 and 450 nm, respectively. Slight differences in lutein content were observed between the three blue LED treatments, but it was not found to be statistically significant as shown in **Fig. 3**.

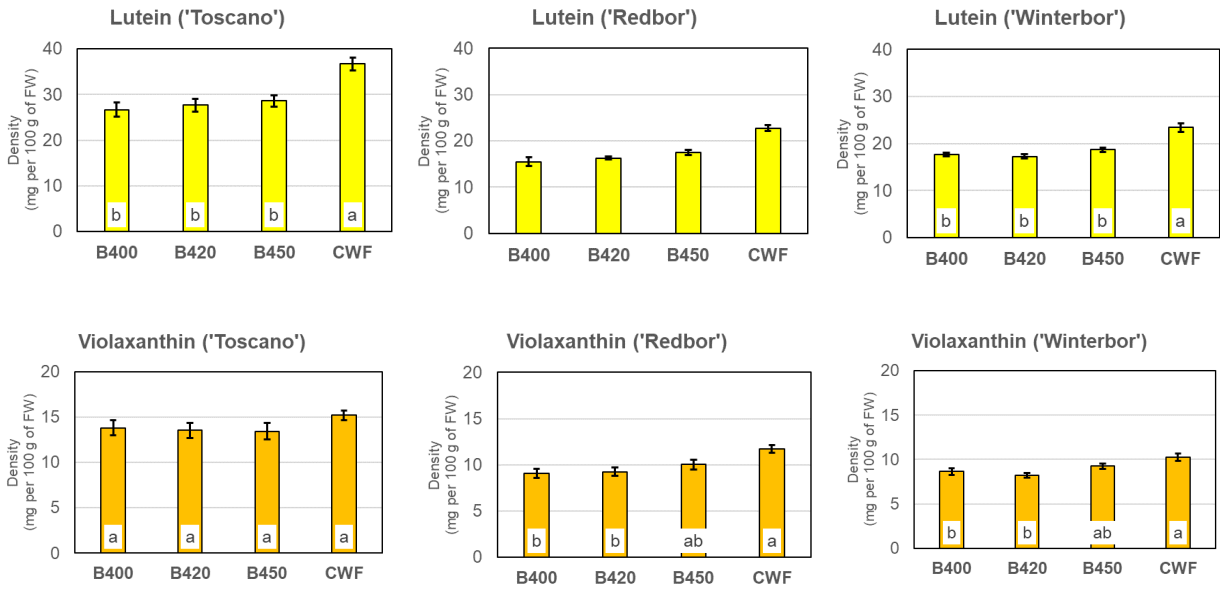


Fig. 3. Carotenoids measurements (lutein and violaxanthin) in the leaves of 'Toscano', 'Redbor', and 'Winterbor' kale plants grown under the three LED and CWF (control) light treatments. Error bars represent mean ± SE from three biological replicates. Sample size per cultivar: *n* = 12. Tukey's significance letters are indicated inside bar plots at the bottom. Zeaxanthin was not detected in any of the leaf samples.
[Two column fitting]

The highest violaxanthin content was found in leaves from B450 and CWF groups (both with higher blue λ_{peak}) for 'Redbor' and 'Winterbor' kale (see **Fig. 3**). Non-statistical difference in violaxanthin content was observed for 'Toscano' kale between the different light treatments. Zeaxanthin was not detected in any of the leaf samples due to low indoor light intensity [30] and indoor grown plants typically have less xanthophyll cycle pigments [41].

For 'Toscano' and 'Winterbor' kale, the highest concentration of chlorophyll *a* was found in leaves harvested from B450 and CWF light treatments (see **Fig. 4**). For 'Redbor' kale, the chlorophyll *a* content from CWF treatment was 61.4 %, 43.7 %, and 28 % higher than the chlorophyll *a* pigment found in the leaves from the B400, B420, and B450 LED treatments. For the three kale cultivars, an increase in lutein and chlorophyll *a* was observed in the blue LED treatments from B400 to B450 (see **Fig. 3 and 4**).

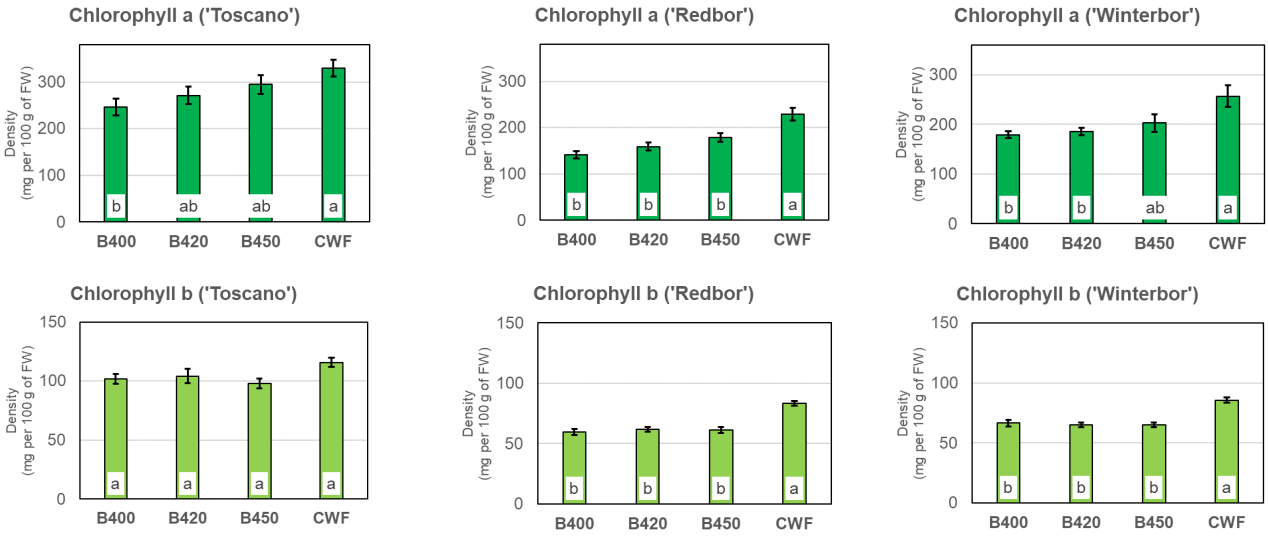


Fig. 4. Chlorophyll measurements (Chl *a* and *b*) in the leaves of 'Toscano', 'Redbor', and 'Winterbor' kale plants grown under three LED and CWF (control) light treatments. Error bars represent mean ± SE from three biological replicates.

373 Sample size per cultivar, *n* = 12. Tukey's significance letters indicated inside
374 bar plots at the bottom. **[Two column fitting]**
375

376 For chlorophyll *b* pigment, the highest density was observed in plants grown
377 under CWF light for 'Redbor' and 'Winterbor' kale (**Fig. 4**). Measured chlorophyll *b*
378 concentration in leaves from the CWF treatment was 40 % and 36 % higher than the
379 pigment concentration in B400 and B450 LED treatments, respectively, in 'Redbor' kale
380 samples. For 'Toscano' kale, the differences in foliar chlorophyll *b* content between the
381 different treatment groups were not statistically significant.

382 Variety-dependent response in pigment content was evident in this study.
383 Overall, the pigment density (lutein, violaxanthin, chlorophyll *a* and *b*) in 'Toscano' kale
384 leaves was higher than pigments in 'Redbor' and 'Winterbor' kale leaves. In terms of
385 total chlorophyll and total carotenoid content, leaf samples grown under CWF light
386 contained significantly higher amounts of these photosynthetic pigments than leaves
387 from the LED treatments (see **Table 2**). Total carotenoids (lutein and violaxanthin) in
388 'Toscano' kale from CWF treatment were 27.9 % and 23.3 % larger than the
389 carotenoids from B400 and B450 treatments, respectively.

390 Between the different blue LED treatments, higher pigment density was observed
391 in leaves from B450 than in the other two treatments, but the difference was not found
392 to be statistically significant. For instance, the total carotenoid content in 'Redbor' leaves
393 from the B450 treatment was 11.8 % and 7.8 % higher than the measured carotenoids
394 'Redbor' leaves from the B400 and B420 treatments, respectively.

The ratio of chlorophyll *a* to chlorophyll *b* (Chl. *a/b*) in leaf samples was observed to be relatively low in this study **(Table 2)**, which is characteristic of plants grown at low light levels [41-43]. For the three kale cultivars, the Chl. *a/b* values from B400 and B420 treatments were slightly lower than the ratios in the B450 and CWF treatments.

Previously, a higher concentration of carotenoids and chlorophyll compounds was reported in leaf samples grown under light treatments with a large percentage of blue light [16,20]. Camejo and co-workers [37] observed higher anthocyanin content (cyanidin) in the CWF light group than in white-LED and red and blue-LED treatments (all at constant PPFD of 250 µmol m^{-2} s^{-1}), likely due to the difference in the blue light spectrum. This trend was also evident in this study with plants grown under the CWF light treatment, which contained a higher blue light fraction (see **Fig. 1**).

Blue light is known to enhance the production of phenolic compounds (such as anthocyanins) in various plant species mediated by cryptochrome and phototropin photoreceptors [44-45]. A higher concentration of phenolic compounds was previously measured in red leaf 'Lollo Rossa' lettuce exposed to longer durations of supplemental blue light [46]. In this study, non-significant differences were observed for total phenolics between treatment groups (see **Supplementary materials**). However, the TPC values in CWF-grown leaves were slightly higher than the TPC values measured from leaves from blue LED treatments. Vastakaite-Kairiene *et al.* [39] observed end-of-day blue light negatively impacted the accumulation of total phenolic compounds in lettuce. Similarly, Li and researchers [40] found no applicable changes in total phenolic compounds of two lettuce cultivars during a 24-d study.

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Table 2: Effect of blue peak wavelength on the phytochemical content in 'Toscano', 'Redbor', and 'Winterbor' kale. Values are reported as mean ± SE. Sample size: n = 12.

A) 'Toscano'

Light treatment	Chl. *a/b* ratio	Total chlorophylls[a]	Total carotenoids[a]
B400	2.4 ± 0.2b	348 ± 20b	40.5 ± 2.0b
B420	2.6 ± 0.1ab	375 ± 24ab	41.0 ± 2.0b
B450	3.0 ± 0.2a	393 ± 23ab	42.0 ± 2.1b
CWF	2.9 ± 0.1ab	446 ± 20a	51.8 ± 1.8a

B) 'Redbor'

Light treatment	Chl. *a/b* ratio	Total chlorophylls[a]	Total carotenoids[a]
B400	2.4 ± 0.1a	201 ± 9b	24.6 ± 1.3b
B420	2.6 ± 0.1a	221 ± 10b	25.5 ± 0.7b
B450	3.0 ± 0.2a	240 ± 10b	27.5 ± 1.0b
CWF	2.8 ± 0.2a	312 ± 14a	34.4 ± 1.0a

C) 'Winterbor'

Light treatment	Chl. *a/b* ratio	Total chlorophylls[a]	Total carotenoids[a]
B400	2.7 ± 0.1a	246 ± 9b	26.4 ± 0.7b
B420	2.9 ± 0.1a	250 ± 9b	25.5 ± 0.6b
B450	3.1 ± 0.2a	268 ± 19b	27.9 ± 0.7b
CWF	3.0 ± 0.2a	342 ± 24a	33.7 ± 1.3a

[a] Total chlorophylls (chlorophyll *a* and *b*) and carotenoids (lutein and violaxanthin) were reported in "mg per 100 g of FW".

3.4. Leaf gas exchange analysis

The exchange of CO_2 and H_2O from leaf samples was measured from each light treatment group at the end of the growth period. Similar to the biomass analysis, instantaneous leaf gas exchange measurements between the different light treatments were found not to be different (see **Supplementary materials**). For 'Toscano' and 'Winterbor' kale, non-significant differences in the parameters, WUE, A_n, E, and gs were observed. For 'Redbor' kale, significantly higher WUE and E values were found in B400 and B420 LED treatments.

Overall, there was a decreasing trend in A_n from B400 to B450, based on the measured data. Leaves from the blue LED treatments had slightly higher A_n than leaves from control CWF lights, an exception with B420 in 'Winterbor' kale. There was large variability in the data likely due to non-saturating, low incident light (200 µmol m^{-2} s^{-1}) during gas exchange measurements. Previously, A_n values of 5 - 10 µmol CO_2 m^{-2} s^{-1} were reported in lettuce leaves grown under LED treatment (16 % Blue + 20 % Green + 64 % Red) and exposed to PPFD of 200 µmol m^{-2} s^{-1}[2], similar to the current findings. At higher irradiance (PPFD > 700 µmol m^{-2} s^{-1}), A_n increased to 15 – 20 µmol CO_2 m^{-2} s^{-1} in leaf samples. Furthermore, Piovene and co-workers [46] did not find significant differences in A_n measurements on basil leaves between LED and fluorescent light treatments (excluding 10 % B + 90 % R LED treatments, which yielded significantly lower A_n).

Higher WUE values are associated with higher PPFD or ambient CO_2 concentrations, while instantaneous WUE measurement depends on many environmental factors such as light and drought conditions [47-48]. Furthermore, A_n measurements from individual leaves or time points in a treatment group may not have a good correlation with crop yield [49]. In basil leaves, gs values of 140 – 220 mmol H_2O m^{-2} s^{-1} (approx.) were reported in leaves from different LED and CWF (control) treatments, with lower gs values, found when the R: B ratio in the light source dropped below 1 [4].

Hogewoning and co-workers [26] found an increase in light-saturated CO_2 assimilation rate (A_{max}), gs, and the number of stomata units (adaxial side) in cucumber leaves when the percentage of blue light was increased from 0 % to 50 % of PPFD. In another study, a higher photosynthetic rate and gs were reported in lettuce leaf samples when growth light had a high blue light fraction [49]. However, a saturation of photosynthetic parameters was evident at the later growth stage (20 days after transplanting), irrespective of the blue light spectrum. Excessive light can, however, induce photoinhibition, which results in a reduction of A_{max} [50].

4. CONCLUSION

Findings from this research demonstrate that the total amount of light available to kale plants under sole-source lighting conditions is more important than light quality for photosynthesis and biomass accumulation. On the other hand, plant morphology and nutrient yield are largely influenced by the spectral quality (blue light fraction) of light. In this work, differences in biomass yield for all three kale cultivars ('Toscano', 'Redbor', and 'Winterbor') between the different light spectra were found to be small and non-significant. This was also evident in gas exchange measurements, as the CO_2 assimilation rate and WUE were similar for the different kale cultivars, irrespective of blue λ_{peak}. From pigment analysis, the concentration of total carotenoids and total chlorophylls was significantly higher in plants grown under CWF light. This is likely due to the higher blue and lower FR light amount in CWF, in comparison to the different blue LED lights. In conclusion, our data indicate that kale plant growth under sole-source lighting is primarily regulated by the total irradiance level, while the accumulation of phytochemicals is controlled by the spectral composition of growth light. This study is one of the first to demonstrate the influence of specific peak emission wavelengths of blue light on plant nutrition.

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