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ORIGINAL ARTICLE

Supplemental light quality affects optimal seeding density of microgreens

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Abstract

Higher seeding densities can negatively affect the yield of microgreens. We quantified the effects of supplemental light quality and seeding density on the yield of microgreens. Seeds of red beet (*Beta vulgaris* L. ssp. *esculenta*) were grown in a greenhouse and provided with a narrow-spectrum (or "purple") and broad-spectrum (or "white") supplemental lighting at nighttime. Purple spectrum contained 90% red, 0% green, 10% blue, and 0% far-red (R_{90}: G_0: B_{10}: FR_0) light, and white spectrum had 35% red, 42% green, 23% blue, and 0% far-red light (R_{35}: G_{42}: B_{23}: FR_0). Each light quality treatment contained seeding densities of 50, 150, 300, and 450 g m^{-2}. We measured fresh weight (FW) and dry weight (DW) on days 8, 10, 12, and 14 after sowing, relative growth rate (RGR), canopy area (CA), and chlorophyll $(chl)_a$, chl_b, betacyanin, and betaxanthin pigments. The optimal seeding density in the white treatment was 300 g m^{-2}, whereas FW increased even at the seeding density of 450 g·m^{-2} in the purple treatment. The RGR of seedlings decreased with increasing seeding density but was higher by 27.8% in the purple than white treatment. The slope of the relationship between DW and CA (related to photosynthetic efficiency) was higher for the purple than white treatment (0.012 vs. 0.008 g cm^{-2}). The ratio of chl_a to chl_b (related to photosynthetic reaction center activity) increased by 15% in the purple than white treatment. Therefore, higher optimal density in the purple than white light quality treatment is likely due to increased photosynthetic efficiency of seedlings.

1 | INTRODUCTION

It is estimated that 2 billion people in the world are affected by hidden hunger, a chronic deficiency of essential minerals and vitamins (Lowe, 2021; WHO, 2009). One reason for this is the unavailability of nutritious food due to economic, edaphic, and climatic factors. Poor diet can lead to several non-transmissible chronic diseases (e.g., cardiovascular, diabetes, etc.) in humans. Consuming plant-based food rich in fiber, minerals, and bioactive compounds can reduce health-related issues in humans (Samtiya et al., 2021; WHO, 2002). Microgreens are the seedlings of vegetable, aromatic, and legume species. They are harvested when the cotyledons are fully expanded with or without the first true leaves (Xiao et al., 2012). Microgreens have gained immense popularity

Abbreviations: CA, canopy area; chl, chlorophyll; DW, dry weight; FR, far-red; FW, fresh weight; LED, light emitting diode; RGR, relative growth rate.

in recent years due to their numerous nutritional benefits, unique flavors, and colors. Therefore, consumption of microgreens can improve human health. Among microgreens, beet is popular due to high levels of nutrients such as betalains (betacyanins and betaxanthins) with high antioxidant activity (Tanaka et al., 2008).

Seeding density is an important attribute affecting the yield of microgreens (Murphy & Pill, 2010; Storey, 2017). Optimal seeding density for microgreens can vary based on species. A study that tested seeding densities of 50, 100, 150, and 200 g· m^{-2} for beet microgreens found that the shoot fresh weight (FW) increased linearly with increasing density (Murphy & Pill, 2010). However, the yield of radish seedlings showed a curvilinear response to increasing seeding densities with a decrease at higher densities (Thuong & Minh, 2020). Generally, increasing seeding density decreases individual seedling weight (Allred & Mattson, 2018), likely due to increased competition for resources. Factors such as light, water, and nutrients can become limiting with increasing seeding density and potentially reduce photosynthesis rate and yield of microgreens. Moreover, higher optimal seeding densities can increase the incidence of diseases in microgreen production (Storey, 2017; Treadwell et al., 2010).

Among other environmental variables, spectral composition of light received by plants can affect photosynthesis (Fang et al., 2021; Kobori et al., 2022; M. Lee et al., 2019; Son & Oh, 2015). The photosynthetic efficiency (fraction of light energy converted into chemical energy) is highest for red light (McCree, 1971). Because of this, higher proportion of red light in the total light results in increased shoot dry weight (DW), chlorophyll (chl) content, and photosynthetic apparatus development (M. J. Lee et al., 2014; Paradiso et al., 2011). Generally, a small percentage of blue light (10%–15%) is recommended in the total light as high levels of blue light decreases crop yield (Kopsell & Sams, 2013; Samuolienė et al., 2017). The photosynthetic efficiency of absorbed green light is relatively lower compared to red but higher than blue (McCree, 1971). However, absorption of green light by chl is lower than that of both red and blue light (McCree, 1971).

Given the effect of light quality on physiological responses in plants, the yield of microgreen seedlings can be influenced by the light quality provided during growth. It is possible that, at a given seeding density, yield can increase or decrease depending on the light quality. For example, a higher seeding density that negatively affects yield in one light quality may have a different response in another light quality with more favorable effects on growth. In other words, recommendations of optimal seeding density (that result in maximum yield) should be based on the light quality provided to microgreen seedlings, when other environmental variables remain constant. This information has potential value to increase the productivity of microgreens in commercial greenhouses and indoor production systems as customized artificial light quality is provided to seedlings using light emitting diodes (LEDs). However, studies that looked at the interaction between light quality and seeding density of microgreens are limited.

Core Ideas

- Microgreens have gained popularity due to their nutritional quality.
- Seeding density influences the yield of microgreens.
- We tested if optimal seeding density is affected by supplemental light quality.
- Optimal seeding density increased when supplemental light had high proportion of red light.
- These results can have potential use in greenhouse/indoor production of microgreens.

In this study, we hypothesized that optimal seeding density of microgreens will depend on light quality and be higher for narrow-spectrum (with high proportion of photosynthetically efficient red light) than broad-spectrum (with low proportion of red and high proportion of blue or green light) light provided to seedlings. The objectives of the study were to (i) quantify the interactive effects of supplemental light quality and seeding density on the yield and (ii) associate the observed differences in yield to the underlying whole-plant physiological responses in greenhouse-grown beet microgreens.

2 | MATERIALS AND METHODS

2.1 | Seeds and seedling growth

The study was conducted during April and May 2021 in a glass greenhouse with temperature control (evaporative cooling pads) and LED supplemental lighting. Organic seeds of red beet (*Beta vulgaris* L. ssp. *esculenta*) were procured from Johnny's Select Seeds and germinated in standard 1020 trays (51 cm × 25 cm × 6 cm) filled with a soilless substrate (80% peat, 15% perlite, and 5% vermiculite, BM-2, Berger). Seeds were also sown in liner trays (17.8 cm × 13.3 cm) at the same time for additional analyses (see Section 2.3). Trays were placed under a mist system until germination. The average temperature and relative humidity during the germination stage were 22.5 (±0.73)°C and 78.7 (±15.34)%, respectively.

After 5 days, germinated trays were transferred to ebb-flow irrigation tables (122 cm × 31 cm × 10 cm; Botanicare) located in a different section of the greenhouse. Seedlings were subirrigated with a water-soluble fertilizer containing 20N-8.7P-16.6K (20-20-20 Jacks Professional, JR Peters Inc.) at an electrical conductivity (a measure of fertilizer concentration) level of 0.7 ± 0.05 dS·m^{-1} and pH 5.8 ± 0.04. The fertilizer solution was stored in reservoirs (76 L capacity; Botanicare) placed below the ebb-flow irrigation system. The fertilizer solution was pumped using submersible pumps (530 L·h^{-1}; Total Pond) connected to the ebb-flow irrigation tables. The pumps were turned on every day for 15 min to provide the fertilizer solution to the seedlings. The substrate in the trays absorbed the fertilizer solution through the bottom holes by capillary action. The excess fertilizer solution drained back into the reservoirs through tubing connecting the outlet of ebb-flow irrigation table and the reservoir. The average daytime and nighttime temperatures in the greenhouse were 22.4 (±2.42)°C and 20.7 (±2.33)°C, respectively, during the study. The average sunlight intensity received by seedlings was 13.0 (±6.57) mol m^{-2} day^{-1} during the study.

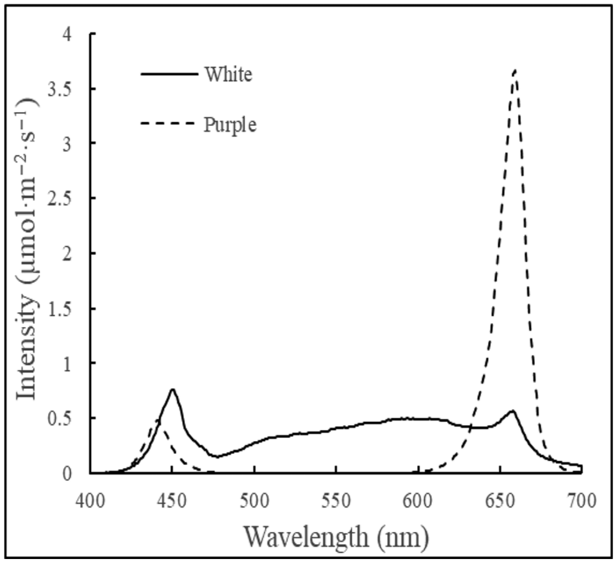

FIGURE 1 Spectral composition of supplemental light provided to beet seedlings at nighttime. The narrow (or "purple") spectrum contained a higher proportion of red light (601–700 nm) than the broad (or "white") spectrum. The proportions of blue (400–500 nm) and green (501–600 nm) light were higher in the "white" than "purple" light quality treatment.

2.2 | Treatments

Beet seedlings were subjected to two different light quality treatments using narrow-spectrum (hereafter "purple") and broad-spectrum (hereafter "white") LED supplemental lights (Ray 44; Fluence Bioengineering). Purple spectrum contained 90% red, 0% green, 10% blue, and 0% far-red (R_{90}: G_0: B_{10}: FR_0) light, and white spectrum had 35% red, 42% green, 23% blue, and 0% far-red light (R_{35}: G_{42}: B_{23}: FR_0). The peak wavelength of red light was 659 nm in both treatments. The supplemental lighting was provided daily during the nighttime from 9 p.m. to 6 a.m. This was done to maximize the light quality effects (in the absence of sunlight) on beet seedlings. Each unit of the light quality treatment contained four LED bars that were 56-cm long and spaced 46 cm apart from each other. The supplemental lighting provided 4.2 and 4.1 mol m^{-2} day^{-1} of additional light to seedlings in the purple and white light quality treatments, respectively. Within each light quality treatment, beet seedlings were grown at four different seeding densities of 50, 150, 300, and 450 g m^{-2}. Each seeding density unit was 25.5 cm × 25 cm (637.5 cm^2) in size. To ensure uniform sowing, each seeding density unit was divided into two sub-units (318.75 cm^2 each), and the seed weight required for each sub-unit was weighed separately and uniformly spread over the substrate.

2.3 | Measurements

Temperature (ST 110, Apogee Instruments) and light (SQ-100, Apogee Instruments) sensors were connected to a datalogger (CR1000; Campbell Scientific) and placed in the middle of each light quality treatment to continuously measure air temperature and light intensity. The spectral composition of the LED lights was measured at the start of the study using a field spectroradiometer (SS110; Apogee Instruments) at nighttime (Figure 1).

As it is difficult to individually separate leaves of all seedlings for measuring total leaf area, an image analysis approach was used to nondestructively estimate canopy area (CA) of seedlings. The seedlings grown in liner trays were placed inside an image station (TopView, Aris) to capture and automatically process images. The automated image processing technique successfully segmented background from the seedlings and estimated CA (cm^2) (Figure 2). Total FW of seedlings was measured on days 8, 10, 12, and 14 after sowing. At each time point, plants were manually harvested using scissors at a height of approximately 1 cm from the substrate. A plastic square with dimensions of 12.5 cm × 12.5 cm was used to delimit a central area to be harvested in each unit. FW of seedlings was immediately measured after harvest using a precision balance. Seedlings were dried in a forced-air oven maintained at 60°C until constant weight to obtain the total DW. The total FW and DW were expressed on a unit area basis (g m^{-2}).

Relative growth rate (RGR, day^{-1}) of seedlings was calculated as follows (Hunt, 1990):

$$\mathrm{RGR} = \frac{\ln\left(\mathrm{DW}_2\right) - \ln\left(\mathrm{DW}_1\right)}{t_2 - t_1}$$

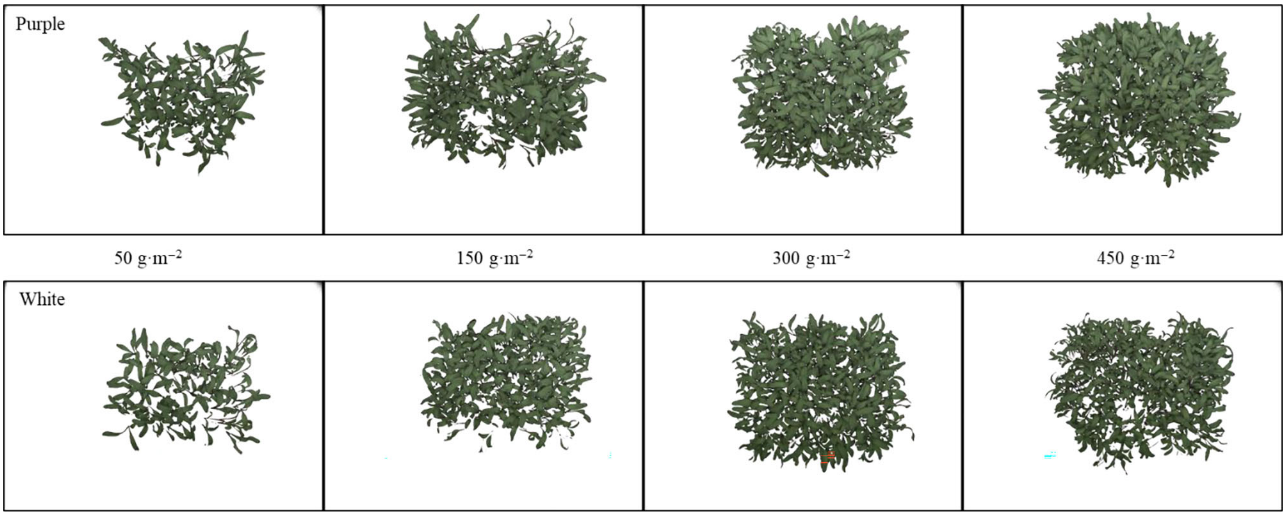

FIGURE 2 An illustration of image analysis used for canopy area measurement in beet microgreens at different seeding densities from "purple" and "white" light quality treatments. Note the accuracy of segmentation by the image analysis software to remove the background from plant pixels.

The DW_2 and DW_1 are total DW of seedlings at times t_2 (day 14) and t_1 (day 8), respectively.

Microgreens samples were collected from liner trays after imaging to perform pigment analysis. Samples were stored in paper envelopes and immediately flash frozen using liquid nitrogen for the assessment of chl, carotenoids, and betalains. Samples were manually ground in a mortar with liquid nitrogen and stored in Eppendorf tubes at −20˚C inside a freezer until the day of each pigment extraction. Chl and total carotenoid extraction was performed using the methodology described by Arnon (1949). A 0.1 g sample was diluted using 1.5 mL of 80% aqueous acetone and stored in a dark place overnight. Then, the tubes were centrifuged (Sorvall Legend Micro 21 Centrifuge, Thermo Scientific) for 20 min at 10,000 rpm, and 0.2 mL of the supernatant was diluted in 0.7 mL of acetone extract solution and shaken in a vortex. The absorbance was measured using a UV-Vis spectrophotometer (Bio Mate 160, Thermo Scientific) at 645 and 663 nm for chlrophylls (chl_a and chl_b, respectively) and 470 nm for total carotenoid analysis. The quantification of the pigments was calculated using the equations proposed by Lichtenthaler and Wellburn (1983). From these values, total chl and chl_a: chl_b were calculated as $chl_a + chl_b$ and $\frac{chl_a}{chl_b}$, respectively.

The methodology proposed by Stintzing et al. (2003) was used for extracting the betalains. A 0.2 g sample was diluted in 1.5 mL of 50% methanol and left in the dark at room temperature for about 30 min. The samples were centrifuged at 10,000 rpm for 30 min, and 1 mL of this extract was added to 0.9 mL of McIlvaine buffer (0.1 M, pH 6.5). The absorbances were read by spectrophotometer at 538, 480, and 600 nm. Betalains concentration (mg 100 g^{-1} of FW) was calculated as the product of $A \times DF \times MW \times V \times \frac{100}{\varepsilon \times l \times W}$, where A is the absorption value at 538 (betacyanin) or 480 (betaxanthin) nm normalized to the absorption at 600 nm, DF is the dilution factor, MW corresponds to the molecular weight (betacyanin: 550 g mol^{-1} and betaxanthin: 340 g mol^{-1}), V is the volume of the pigment solution (L), ε is the molar absorptivity (betacyanin: 60,000 L mol cm^{-1} and betaxanthin: 48,000 L mol cm^{-1}), l is the cuvette thickness, and W is the DW of the plant material (g).

2.4 | Experimental design and statistical analysis

The study used a split-plot design with two light quality treatments (main plots) and four seeding densities (split plots). The main plots were replicated four times. Main and split plots were randomly allotted in each replication. Data were analyzed according to a mixed linear model ("Proc Mixed" procedure) with repeated measures using Statistical Analysis Software (SAS Institute Inc.). Least square means were separated using Tukey's honestly significant difference test with $p \leq 0.05$ considered statistically significant. Regression analysis was conducted by fitting linear responses using the "Proc Reg" procedure of SAS with $p \leq 0.05$ considered statistically significant for slope. Graphs were plotted using Microsoft Excel software.

3 | RESULTS

3.1 | Fresh and dry weights

The three-way interaction among light quality, seeding density, and time of harvest on FW of beet seedlings was significant (Figure 3). On the 8th, 10th, and 12th day after

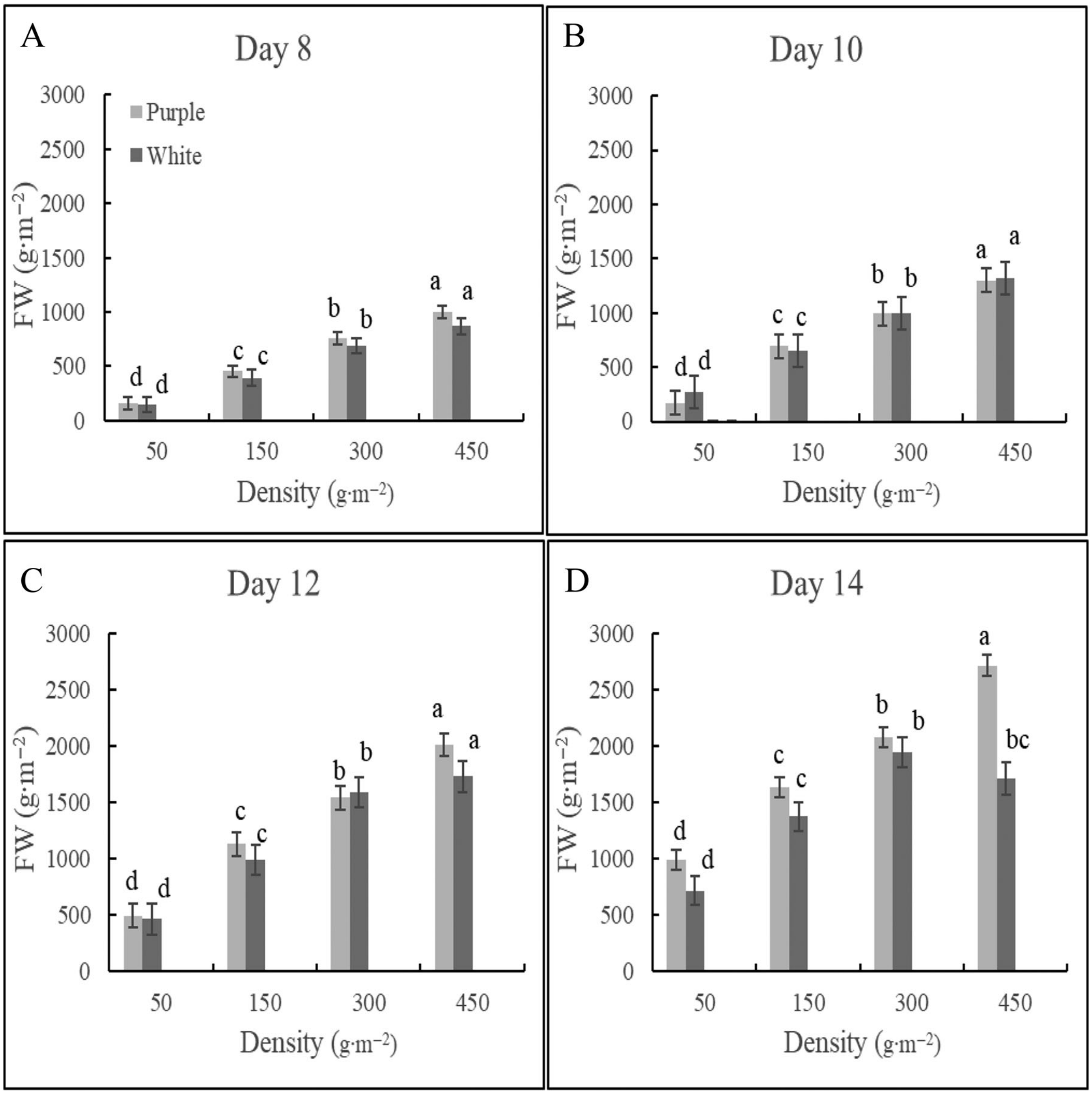

FIGURE 3 Three-way interaction among light quality, seeding density, and harvest at Days 8 (A), 10 (B), 12 (C), and 14 (D) on fresh weight of beet seedlings. Tukey mean ($n = 4$) separation is shown using different letters for comparing the interactive effects light quality and seeding density at a given harvest time on fresh weight of beet seedlings. Least square means separated by different letters are statistically different. FW, fresh weight.

sowing, the FW of beet seedlings increased with increasing seeding density without any statistical differences between the two light quality treatments (Figure 3A–D). On all the harvest days except day 14, the FW of seedlings at a density of 300 g·m^{-2} was lower than 450 g·m^{-2} but higher than that of 150 g·m^{-2} and that of 150 g·m^{-2} was higher than 50 g·m^{-2}. A nonsignificant decline in FW was observed at a seeding density of 450 g·m^{-2} in the white than purple light quality treatment on the 12th day after sowing (Figure 3C). However, FW significantly decreased (approximately 30%) in the white (1712 g m^{-2}) compared to the purple (2713 g m^{-2}) light quality treatment at a seeding density of 450 g·m^{-2} on the 14th day after sowing (Figure 3D). The FW was not statistically different between 450 and 300 g·m^{-2} seeding density treatments in the white, whereas it significantly increased during the same time in the purple (2713 vs. 2076 g m^{-2}) light quality treatment on the 14th day after sowing (Figure 3D). Statistical analysis indicated that the response of DW to light quality, seeding density, and time of harvest was identical to that of FW. A near perfect linear and positive relationship was observed between FW and DW of beet microgreens (DW = 0.063 × FW, r^2 = 0.98; data not shown). Based on the slope of the fitted equation, DW increased by 6.3 g for every 100 g increase in FW or DW accounted for 6.3% of FW.

3.2 | Relative growth rate

The interaction between light quality and seeding density was not significant, but there were significant main effects of

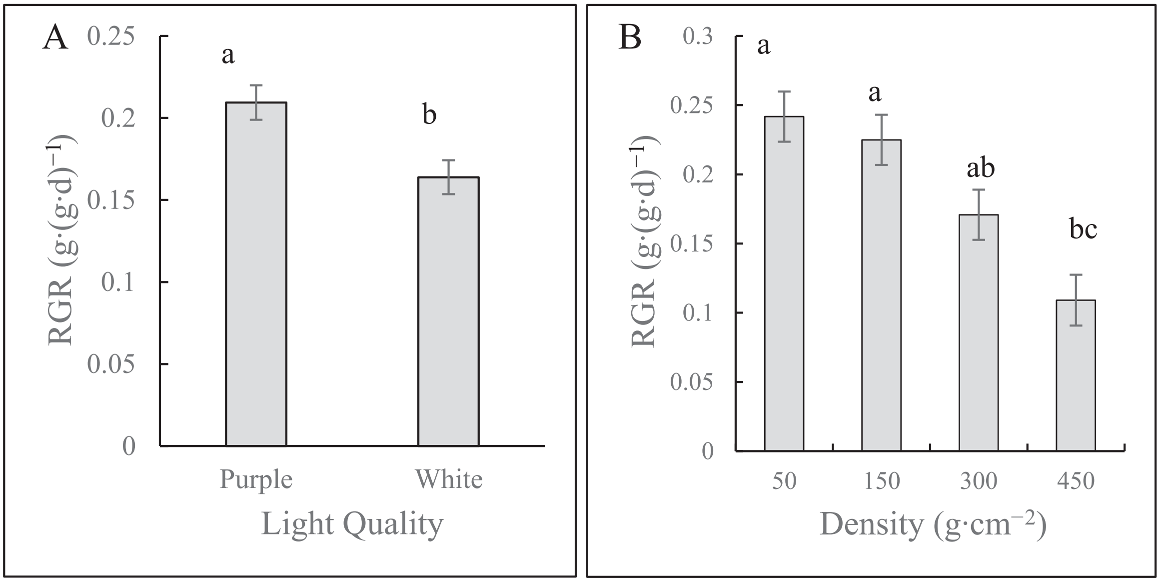

FIGURE 4 Main effects of light quality (A) and seeding density (B) on relative growth rate (RGR) of beet seedlings between days 8 and 14 after sowing. Least square means separated by different letters are statistically different.

light quality and seeding density on RGR of beet seedlings. The values of RGR were significantly higher in the purple (0.21 g [g day]$^{-1}$) than white (0.16 g [g day]$^{-1}$) light quality treatment, when averaged across all four seeding densities (Figure 4A). There was approximately 28% increase in RGR in the purple compared to white light quality treatment. Statistical analysis indicated that RGR was not different between the densities of 50 (0.24 g [g day]$^{-1}$) and 150 (0.23 g [g day]$^{-1}$) g·m^{-2}, intermediate in the 300 g·m^{-2} (0.17 g [g day]$^{-1}$) treatment, and lowest in the 450 g·m^{-2} (0.11 g [g day]$^{-1}$) treatment (Figure 4B). The RGR was higher by approximately 121% at 50 compared to 450 g·m^{-2} seeding density.

3.3 | Canopy area

The CA of beet microgreens was significantly affected by seeding density, while light quality did not show any significant effect on CA. Averaged across both light treatments, the CA of beet seedlings was highest and not different between 450 g·m^{-2} (183 cm^2) and 300 g·m^{-2} (168 cm^2), followed by 150 g·m^{-2} (133 cm^2), and lowest at a seeding density of 50 g·m^{-2} (74 cm^2) (Figure 5A). There was approximately 80% increase between 150 and 50 g·m^{-2}, 26% increase between 300 and 150 g·m^{-2}, and nonsignificant change between 450 and 300 g·m^{-2} in CA. The image-derived measurements of CA were closely related to total FW of beet seedlings when data were pooled from both light treatments and four seeding densities (Figure 5B).

There was a linear and positive relationship between total DW and CA of seedlings when data from all seeding densities were pooled separately for white and purple light quality treatments (Figure 6). Total DW of beet seedlings increased with increasing CA in both light quality treatments. However, regression analyses indicated that the slope of the relationship between DW and CA was higher (approximately 45%) in the purple than white light quality treatment.

3.4 | Pigments

Analyses of different pigments showed no significant effects of light quality and seeding density on total chl, total carotenoid, and betaxanthin levels in beet microgreens (Table 1). However, a main effect of light quality on betacyanin (Table 1) and main effects of light quality and seeding density on chl$_a$:chl$_b$ were observed (Figure 7A,B). Betacyanin levels were significantly higher in the purple (10.1 mg 100 g^{-1}) than white (8.9 mg 100 g^{-1}) light quality treatment with an increase of 13% (Table 1). In general, chl$_a$ was more than chl$_b$ by a factor of 4.6–5.1 among different light quality and seeding density treatments. The chl$_a$:chl$_b$ value was significantly higher in the purple (4.81) than white (4.74) light quality treatment by approximately 1.5% (Figure 7A). The chl$_a$:chl$_b$ value was higher at a seeding density of 50 g·m^{-2} (5.04) than 150 g·m^{-2} (4.83). It was not statistically different in the two higher seeding densities of 300 and 450 g·m^{-2} (Figure 7B). The ratio decreased by approximately 8.5% at a seeding density of 450 g·m^{-2} compared to that at 50 g·m^{-2}.

4 | DISCUSSION

In our study, the optimal seeding density was 300 g·m^{-2} in the white light quality treatment (Figure 3D). However, optimal seeding density could not be established in the purple

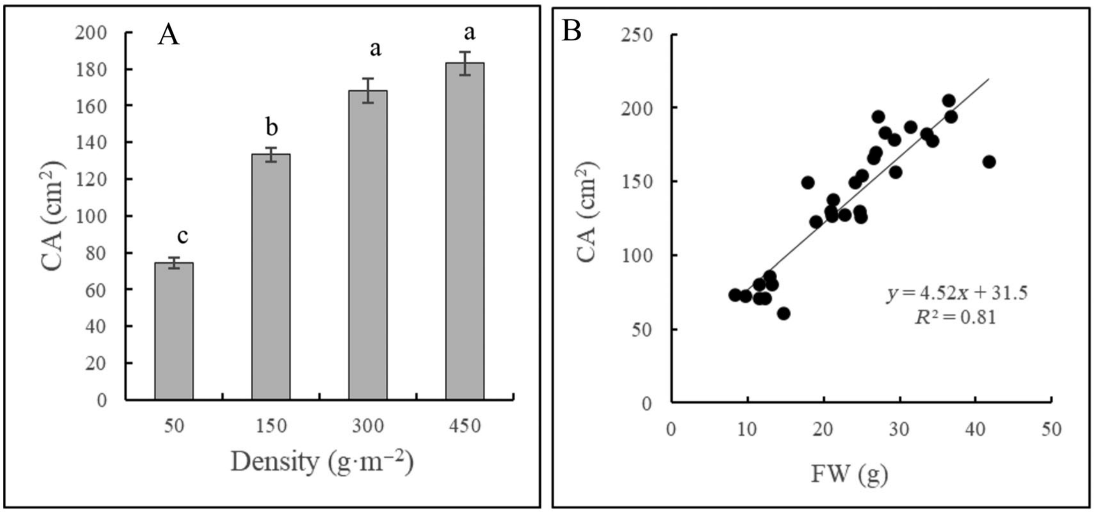

FIGURE 5 (A) Main effect of seeding density on canopy area (CA) of seedlings. The data were pooled from both light quality treatments after 14 days of sowing. Least square means represented by different letters are statistically different. (B) Linear relationship between CA and fresh weight (FW) of beet seedlings. The data were pooled from two light qualities and four seeding densities after 14 days of sowing. The fitted equation was CA = 31.5 + 4.52 × FW (r^2 = 0.81).

TABLE 1 Effects of light quality and seeding density on total chlorophyll (Chl) content, chlorophyll-a to chlorophyll-b ratio (chl_a: chl_b), total carotenoid, betacyanin, and betaxanthin in beet seedlings.

Light quality	Seeding density ($g\ m^{-2}$)	Chl ($mg\ g^{-1}$)	Total carotenoid ($mg\ g^{-1}$)	Betacyanin ($mg\ 100\ g^{-1}$)	Betaxanthin ($mg\ 100\ g^{-1}$)
Purple	50	0.42 (0.017)	0.12 (0.004)	9.0 (0.73)	3.2 (0.25)
	150	0.43 (0.007)	0.12 (0.003)	11.2 (0.18)	3.7 (0.24)
	300	0.44 (0.027)	0.12 (0.004)	9.5 (0.54)	3.2 (0.14)
	450	0.47 (0.008)	0.12 (0.003)	10.9 (0.51)	3.6 (0.23)
White	50	0.46 (0.015)	0.12 (0.002)	8.1 (0.55)	3.0 (0.08)
	150	0.48 (0.011)	0.13 (0.004)	8.2 (0.63)	3.1 (0.39)
	300	0.45 (0.009)	0.12 (0.002)	10.3 (0.26)	3.5 (0.14)
	450	0.44 (0.009)	0.12 (0.002)	9.4 (0.28)	3.5 (0.12)
Light		n.s.	n.s.	0.033	n.s.
Density		n.s.	n.s.	n.s.	n.s.
Light × density		n.s.	n.s.	n.s.	n.s.

Note: Mean followed by standard error in parenthesis are shown.
n.s. denotes not significant at $p \leq 0.05$.

light quality treatment as the FW continued to increase even at 450 g·m^{-2} seeding density treatment (Figure 3D). It is well known that red light improves leaf extension, shoot DW, chl, and photosynthetic apparatus development (M. J. Lee et al., 2014; Paradiso et al., 2011). A high proportion of blue light decreases crop yield but enhances the biosynthesis of flavonoids, anthocyanins, and carotenoids in baby leaf lettuce (Q. Li & Kubota, 2009). Moreover, the photosynthetic efficiency (fraction of light energy converted into chemical energy) of absorbed green light is relatively lower compared to red but higher than blue, although absorption of green light is lower than that of both red and blue light (McCree, 1971). The purple light quality treatment contained a higher percentage of red light, lower percentage of blue light, and no green light compared to the white light quality treatment (Figure 1). These results indicate that the optimal seeding density of microgreens can be higher when spectral quality of provided light results in favorable effects on photosynthesis and vegetative growth. The betacyanin levels increased in the purple light quality, while the levels of other pigments were not affected either by the light quality or seeding density treatments (Table 1). Previously, increased betacyanin levels in red

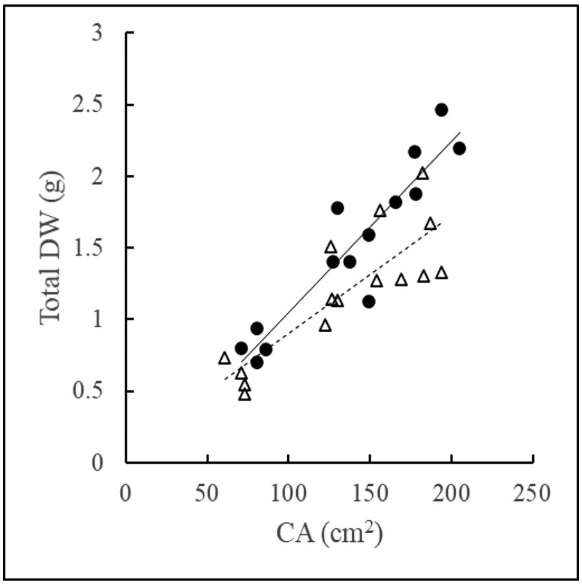

FIGURE 6 Linear relationship between total dry weight (total DW) and canopy area (CA) of beet seedlings provided with "purple" (closed circles and solid line) and "white" (open triangles and dashed line) supplemental lighting. Data from all four seeding densities are pooled. The fitted equations were Total $DW_{purple} = -0.14 + 0.012 \times$ CA ($r^2 = 0.86$) and Total $DW_{white} = 0.09 + 0.008 \times$ CA ($r^2 = 0.71$).

light quality were reported (El-Ashry et al., 2020; Oh et al., 2022) and related to increased availability of photosynthetic assimilates for biosynthesis of betacyanin pigment. Based on our results, beet microgreens should be grown at higher than normally used seeding rates when narrow-spectrum lighting with a higher proportion of red light is used.

A direct relationship between FW and CA of beet seedlings (Figure 5B) and accurate assessments of CA using image analysis (Figure 2) suggest that image-based CA measurements can be used to indirectly and rapidly estimate FW. Further, noninvasive and rapid estimates of CA can be used to study the effect of seeding density rates on yield of different microgreen species. C. Li et al. (2020) used noninvasive measures of CA to estimate biomass and RGR of tomato, basil, lettuce, and zinnia plants. Their study shows that the image-based estimates of CA can be used to accurately monitor growth and adjust inputs during production. Current study further expands the use of image-based CA measurements to rapidly predict growth and yield of microgreens prior to physical harvest.

Assessments of RGR are useful for comparing growth rates of plants differing in size (Boyer, 1982; Hunt, 1990; C. Li et al., 2020; Pommerening et al., 2023), as in our study with beet microgreens grown in different light quality and seeding density treatments. It is known that plants with higher RGR are more efficient at producing new biomass (C. Li et al., 2020). The lower RGR with increasing seeding density in our study indicates that relatively less biomass was produced per day at higher than lower seeding densities. For example, the difference in FW between 150 and 50 $g \cdot m^{-2}$ was 72%, whereas that between 450 and 300 $g \cdot m^{-2}$ was 33% on the 14th day after sowing in the purple light quality treatment (Figure 3D). The differences were more pronounced in the white light quality treatment. There was a 92% increase in FW between 150 and 50 $g \cdot m^{-2}$ and nonsignificant change between 450 and 300 $g \cdot m^{-2}$ (Figure 3D). The higher FW difference observed between 450 and 300 $g \cdot m^{-2}$ seeding densities in the purple light quality treatment on the 14th day after sowing (Figure 3D) can be related to the higher RGR of seedlings in this light quality treatment (Figure 4A). The RGR values observed in our study for beet microgreens are similar to those published for other species such as basil and lettuce (Li et al., 2020).

As RGR is the product of leaf area ratio (related to light interception) and unit leaf rate (related to photosynthesis rate) (Hunt, 1990), changes in both light absorption and/ or photosynthetic efficiency can affect RGR of plants. Light absorption by plants is mostly accomplished by chl (Ouzounis et al., 2015), with chl_a present mainly in the reaction complex of photosystems (Evans, 1989) and chl_b in the light harvesting complex (Kume et al., 2019; Terashima & Hikosaka, 1995). The ratio of chl_a to chl_b has been widely used to assess the extent of shade or low light stress experienced by plants (Gong et al., 2015; Kume et al., 2019; Valladares & Niinemets, 2008). In general, a decline in chl_a: chl_b is observed when plants experience low light conditions from shading (Valladares & Niinemets, 2008). This is due to relatively higher levels of chl_b biosynthesis in the light harvesting complexes of plants to aid in intercepting more-light under low-light conditions. Therefore, a decline in chl_a:chl_b with increasing seeding density (Figure 7B) may suggest that beet seedlings likely experienced shade conditions, regardless of light quality at higher seeding densities. A decrease in RGR of plants due to shading was previously reported (Rees, 2010), especially when acclimation in leaf area ratio is minimal. It is likely that the decrease in RGR with increasing seeding density in our study is in part associated with stress from shading or low light at higher densities. Similarly, a higher chl_a:chl_b in the purple than white light quality treatment may be related to relatively less stress from low light conditions in the purple than in the white light quality treatment. This also indicates that the higher RGR in the purple compared to the white light quality treatment is in part due to higher chl_a:chl_b resulting from relatively less low light stress.

A higher slope for the relationship between total DW and CA in the purple (0.0119 g cm^{-2}) than white (0.0082 g cm^{-2}) light quality treatment (Figure 6) indicates that a unit increase in CA resulted in higher DW in the purple than in the white light quality treatment. This can be interpreted indirectly as higher photosynthetic efficiency of plants in the purple than white light quality treatment as dry matter produced per unit

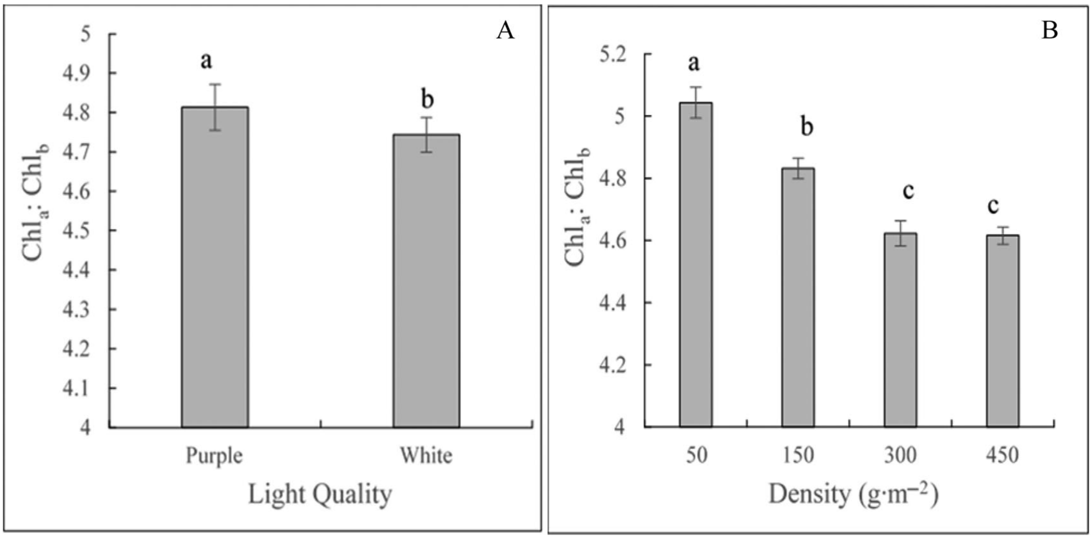

FIGURE 7 Main effects of light quality (A) and seeding density (B) on the ratio of chlorophyll-a to chlorophyll-b (chl_a:chl_b) in beet seedlings. Least square means separated by different letters are statistically different.

leaf area in a given time interval is associated with assimilation rate of plants (Hunt, 1990). Increased photosynthetic efficiency in plants provided with a high proportion of red light in the total light is known since McCree (1971). Higher photosynthetic efficiency may also, in part, be related to higher chl_a: chl_b in the purple than while light quality treatment. An increased demand for energy transfer is needed for higher photosynthetic efficiency and carbon assimilation (Rees, 2010). This will likely require an increased reaction center activity and higher levels of chl_a in the reaction centers, or increased chl_a: chl_b. Therefore, higher photosynthetic efficiency is the likely reason for higher chl_a: chl_b in the purple than white light quality treatment. Collectively, physiological acclimation of seedlings to purple light quality resulted in an increase chl_a: chl_b for supporting increased photosynthetic efficiency of seedlings. This likely lead to higher RGR and lower light stress at higher seeding densities and higher fresh biomass production, compared to those in white light quality treatment.

5 | CONCLUSIONS

Our study demonstrates that the optimal seeding density of beet microgreens is affected by light quality and higher for narrow or purple (with high proportion of red and low proportion of blue light) than broad or white (with low proportion of red and high proportion of blue and green light) spectrum provided to seedlings. Physiological analysis indicated that seedlings in the purple light quality treatment had higher photosynthetic efficiency, chl_a: chl_b, and RGR when averaged across seeding densities. The results from our study can be used to optimize seeding density for given artificial light quality provided to microgreens during production.

AUTHOR CONTRIBUTIONS

Isabela Scavacini de Freitas: Conceptualization; formal analysis; investigation; methodology; validation; writing—original draft; writing—review and editing. **Simone da Costa Mello**: Conceptualization; funding acquisition; methodology. **Krishna Nemali**: Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; software; supervision; validation; visualization; writing—original draft; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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