Food Research 6 (Suppl. 4) : 65 - 70 (2023)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Effect of different LED wavelengths on growth, chlorophyll and anthocyanin content of purple-red pakchoi (*Brassica rapa chineensis)* in indoor vertical farming

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Article history:
Received: 2 April 2022
Received in revised form: 14 May 2022
Accepted: 2 August 2022
Available Online: 17 March 2023

Keywords:
LED light,
Vertical farming,
Controlled environment agriculture,
Sustainable agriculture

DOI:
https://doi.org/10.26656/fr.2017.6(S4).009

Abstract

Understanding the effects of different light wavelengths on plant growth and the accumulation of essential nutrients like antioxidants is critical for obtaining optimal light conditions for the production of high-quality vegetables in indoor vertical farming. Purple-red pakchoi (*Brassica rapa chineensisis*) is a fast-growing brassiceous vegetable. This vegetable is known to have health-promoting properties due to anthocyanin, which is a crucial antioxidant in the diet. This crop was used to evaluate the effects of red (RL), blue (BL), and green (GL) wavelengths on plant morphology, relative chlorophyll content, and phytochemical accumulation (anthocyanin). Light treatments consisted of three different wavelength combinations: a) full spectrum, white light (WL) as a control (the proportions of RL, BL and GL wavelengths were 42%, 15% and 43%, respectively); b) combination $RL_{81}BL_{19}$ (the proportions of RL and BL wavelengths were set at 81% and 19%, respectively); and c) combination $RL_{70}BL_{21}GL_9$ (the proportions of RL, BL and GL wavelengths were set at 70%, 21% and 9%, respectively). The experiment was carried out in the LED Light Research Lab (MARDI, Serdang), a walk-in chamber with a 12-hour photoperiod. The temperature, CO_2, and relative humidity levels in the LED light lab were kept under control. Plant width and height were found to be most prominent in plants exposed under $RL_{81}BL_{19}$ LED light, followed by $RL_{70}BL_{21}GL_9$, while plants grown under WL were relatively small. Purple-red pakchoi exposed to a combination of RL and BL, supplemented with GL light treatment, had a slightly smaller plant height and width than plants exposed to $RL_{81}BL_{19}$ light treatment. Similar results were obtained in terms of shoot fresh and dry weight, with the $RL_{81}BL_{19}$ having the highest shoot fresh and dry weight, followed by the $RL_{70}BL_{21}GL_9$ and WL light exposure plants. Furthermore, when compared to other treatments, purple-red pakchoi treated with $RL_{81}BL_{19}$ light had a significantly higher content ($p<0.05$) of chlorophyll and anthocyanin. This finding indicated that GL light treatment has no direct effect on overall growth, chlorophyll accumulation, or anthocyanin content in purple-red pakchoi. This study found that the $RL_{81}BL_{19}$ wavelength is the best LED light treatment for purple-red pakchoi production in indoor vertical farming.

1. Introduction

Vertical farming is one of the latest technologies offered to produce agro-crops efficiently and has been encouraged by the government. This potential advanced controlled-environment of the cultivation system offers a solution for vegetable production, particularly in land scarcity, labour intensive, pest and disease, and unpredictable weather conditions. In Malaysia, the population will increase to 41.2 million by the year 2040, thus an effective and sustainable vegetable production technology such as vertical farming is needed to reduce the country's dependence on imported vegetables and ensure national food security. Nowadays, the use of LED lights in controlled-environment agriculture practices such as growth chambers, greenhouses, and vertical farming systems is highly encouraged. LED is an abbreviation for light-emitting diode. A diode is an electrical device that consists of two electrodes (an anode and a cathode). LEDs are increasingly being installed in newly built vertical farms (Mickens *et al*., 2019).

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eISSN: 2550-2166
Published by Rynnye Lyan Resources

Vegetables are one of the significant food crops that provide essential nutrients to the Malaysian daily diet (Tee, 1979). Malaysian people like to consume vegetables in raw (as salads) or cooked form. Purple-red pakchoi is widely consumed in Malaysia as it contains a high level of bioactive secondary metabolites such as ascorbic acid and phenolic compounds, which possess high antioxidant activity (Vale *et al*., 2014). The present study evaluated the growth and quality of purple-red pakchoi under LED light incorporated with hydroponic indoor vertical farming. Different mixing ratios of LED were used to examine the plants' growth performance. Purple-red pakchoi was selected as the experimental crop. It is one of the brassiceous vegetables with rapid growth and is enriched with anthocyanin, a powerful antioxidant for dietary consumption (Neugart *et al.,* 2018). Previous studies found that pakchoi has a positive response in growth and secondary metabolite accumulation towards the light treatment given (Son and Oh, 2013; Heng *et al.,* 2018). Specifically, the study aimed to determine the effect of red, blue and green light wavelengths on purple-red pakchoi growth performance and yield quality. The optimisation of light wavelength proportion is crucial for purple-red pakchoi cultivation growth in indoor vertical farming.

2. Materials and methods

2.1 Plant materials and growing conditions

Seeds of purple-red pakchoi (*Brassica rapa chineensis*) were sown in wet urethane foam, which contained 117 cells. The seeds were grown in the hydroponic germination rack with controlled environment conditions using white light, photosynthetic photon flux (PPF) 150, 8 hrs photoperiod for 7 days. The seedlings grown in the urethane foam cube cells were filled into hydroponic pots before being transplanted into multilayer hydroponic system. Environmental conditions in the LED light lab were controlled to maintain the temperature (21.6 to 23.4°C), CO_2 (480 – 497 ppm) and relative humidity (68 - 78%) and were monitored by Temp/RH/CO_2 meters (3445, Spectrum Technologies, Inc., IL, USA). The plants were sub-irrigated as needed with nutrient solution containing (150 mL of nutrient solution/30 L of water). The nutrient solution was supplied to plants continuously until the experiment was completed.

2.2 Light-quality treatments

The study followed a complete randomised design with the lighting environment as the main factor. The light source used in this experiment was LED lights consisting of white, blue, red and green LEDs (Impressive Edge Inc, Malaysia). The experimental conditions were set up with three different treatments. The LED arrays were; full spectrum, which is white light (WL) as a control (the proportions of RL, BL and GL wavelengths were 42%, 15% and 43%, respectively), the combination $RL_{81}BL_{19}$ (the proportions of RL and BL wavelengths were 81% and 19%, respectively) and $RL_{70}BL_{21}GL_9$ (the proportions of RL, BL and GL wavelengths were 70%, 21% and 9%, respectively). The selection of these light treatments was made according to the combination of wavelengths commonly used in the indoor farming industry especially white light (WL), that assigned as the control treatment and combinations of blue and red wavelengths. The combination of blue-red light is commonly used in the indoor farming industry. While the combination of blue: red: green is rarely used, there are some growers who use it. There were seven replicates for each light treatment on each specific harvest day (17, 27, and 37 days after sowing, DAS). A total of twenty-one experimental plants were subjected to each light treatment. The photoperiod was 12 hrs day/12 hrs night for 37 days for each study. Table 1 summarises the output of each treatment in terms of total photosynthetic photon flux density (PPFD) and spectral composition. The spectral distribution scans of all treatments were measured at five locations in the plant-growth area using a spectrometer (LI-180; LI-COR Biosciences, Lincoln, USA).

2.3 Plant morphology and growth characteristics

Plant measurements were performed at 17, 27 and 37 DAS. Growth characteristics included plant width, plant height, fresh weight of shoots, dry weight of shoots, root weight, and total leaf area. Plant height was determined by measuring the vertical distance from the base of the stem to the end of the longest leaf on each plant with a ruler. Plant width was obtained by measuring the horizontal distance between the two most extended leaves. The shoots and roots were dried in a drying oven (UN 30; Memmert, Schwabach, Germany) for 72 hrs at 70°C. The fresh and dry weights from each harvest were obtained using a scale (ME 3002; Mettler Toledo, Ohio, USA). Total leaf areas were measured using a leaf area meter (LI-3100; LI-COR Biosciences, Lincoln, USA).

2.4 Determination of chlorophyll and anthocyanin content

A portable chlorophyll meter (SPAD-502DL; Konika Minolta Sensing, Osaka, Japan) was used to measure relative chlorophyll content in dimensionless SPAD units. Measurements were recorded from three different leaves per plant and the average of the three measurements was recorded. Anthocyanin levels of leaves were estimated non-destructively by being directly exposed to light, using an anthocyanin content

https://doi.org/10.26656/fr.2017.6(S4).009

Table 1. Summary of spectral data for different light treatment

Measurement	Treatment 1	Treatment 2	Treatment 3
Photon Flux Spectral Composition	WL	$RL_{81}BL_{19}$	$RL_{70}BL_{21}GL_9$
Average PPFD (400-700 nm)	133.74	466.58	397.38
Blue (400-500 nm)	20.27 (15%)	87.05 (19%)	85.46 (21%)
Green (500-600 nm)	56.81 (43%)	1.90 (0%)	35.04 (9%)
Red (600-700 nm)	55.86 (42%)	377.72 (81%)	277.20 (70%)
Far-red (700-800 nm)	0.058	0.000	0.001
Ultraviolet (350-400 nm)	0.006	0.000	0.000

Spectra were recorded and averages at five locations at the height of plant canopy with spectrometer at 37 days after sowing. WL: White Light, RL: Red light, BL: Blue light, GL: Green Light, PPFD: photosynthetic photon flux density

meter (ACM-200 plus; Opti-Sciences. Inc., USA). The anthocyanin measurement on plant leaves was carried out during harvests at 17, 27, and 37 DAS.

2.5 Statistical analysis

Statistical analysis was performed using SPSS 23.0 (IBM, Inc., Chicago, IL, USA). All data were analysed for significance by an analysis of variance (ANOVA), followed by Tukey's test for mean separation at the $p<0.05$ (n = 7). Data were tested for normality of residuals and homogeneity of variance prior to carrying out ANOVA.

3. Results

3.1 Effects of light treatments on morphology and growth characteristics

Plants exposed under $RL_{81}BL_{19}$ and $RL_{70}BL_{21}GL_9$ light treatments were observed to have faster growth performance than WL light treatment, which was relatively small (Figure 1). The plants' height and width of purple-red pakchoi under $RL_{81}BL_{19}$ and $RL_{70}BL_{21}GL_9$ treatments were significantly higher ($p<0.05$) than the plants exposed to WL treatment (Table 2) with the largest plant width (31.73 cm, 40.32 cm and 38.17 cm, according to 17, 27 and 27 DAS respectively) and height (13.38 cm, 20.53 cm and 18.95 cm, according to 17, 27 and 27 DAS respectively) observed in the plants that exposed under $RL_{81}BL_{19}$ throughout different DAS period. There was no significant difference ($p>0.05$) in the plant width, height, and leaf area between $RL_{81}BL_{19}$ and $RL_{70}BL_{21}GL_9$ light treatment$_s$ after 37 DAS.

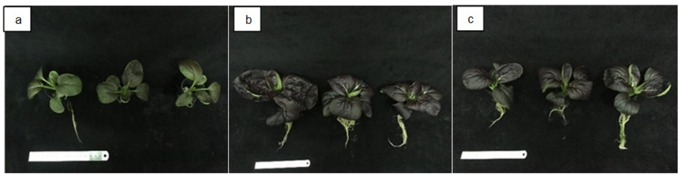

Figure 1. Morphology of purple-red *brassica* under all light treatments (a)WL, (b) RL81BL19 (c RL70BL21GL) at 27 days after sowing (DAS)

The light exposure using $RL_{81}BL_{19}$ and $RL_{70}BL_{21}GL_9$ significantly ($p<0.05$) induced a higher leaf area when compared with WL (control). The highest leaf area was noted in plants treated with $RL_{81}BL_{19}$ (27.96 cm^2, 107.04 cm^2 and 92.31 cm^2, according to 17, 27 and 27 DAS, respectively) even though there was no significant ($p>0.05$) difference with $RL_{70}BL_{21}GL_9$ treatment. The fresh and dry weight of shoots also exhibited significantly ($p<0.05$) increase when exposed to $RL_{81}BL_{19}$ and $RL_{70}BL_{21}GL_9$ treatments (Table 3). The shoots' fresh weight of purple-red pakchoi under $RL_{81}BL_{19}$ (88%) and $RL_{70}BL_{21}GL_9$ (83%) at 37 DAS treatment was higher than that of WL (control). It showed that plants exposed to $RL_{81}BL_{19}$ treatment had the highest fresh (7.94 g, 71.40 g and 48.96 g, according to 17, 27 and 27 DAS, respectively) and dry weight (0.62 g, 5.06 g and 3.38 g, according to 17, 27 and 27 DAS respectively). In terms of root weight, there was no significant difference ($p>0.05$) between treatments on the

Table 2. Influence of LED light combination on plant width, plant height and leaf area of purple-red pakchoi.

Parameters	Plant width (cm)			Plant height (cm)			Leaf area (cm)		
Treatment	17 DAS	27 DAS	37 DAS	17 DAS	27 DAS	37 DAS	17 DAS	27 DAS	37 DAS
WL (control)	7.78^a	21.85^c	31.73^c	4.22^c	9.92^c	13.38^c	3.795^c	8.05^c	27.96^c
$RL_{81}BL_{19}$	10.93^a	33.5^a	40.32^a	7.23^a	17.05^a	20.53^a	8.12^a	42.85^a	107.045^a
$RL_{70}BL_{21}GL_9$	10.52^a	30.95^{ab}	38.17^{ab}	6.85^{ab}	14.22^b	18.95^{ab}	6.37^{ab}	18.56^{bc}	92.32^{ab}
SEM	1.266	1.759	1.227	0.594	0.697	0.506	1.127	5.977	6.162
P	0.195	0.001	0.000	0.005	0.000	0.000	0.049	0.003	0.000

Values with different superscripts are significantly different at the p = 0.05 level by Tukey's multiple comparisons test. Light treatment abbreviation as shown in Figure 1.

https://doi.org/10.26656/fr.2017.6(S4).009

Table 3. Influence of LED light combination on shoot fresh weight, shoot dry weight, root fresh weight and root dry weight of purple-red pakchoi over time

Parameters	Shoot fresh weight (g)			Shoot dry weight (g)			Root fresh weight (g)			Root dry weight (g)		
Treatment	17 DAS	27 DAS	37 DAS	17 DAS	27 DAS	37 DAS	17 DAS	27 DAS	37 DAS	17 DAS	27 DAS	37 DAS
WL (control)	0.27^{c}	10.47^{c}	70.94^{c}	0.03^{c}	0.23^{c}	0.62^{c}	0.11^{c}	0.98^{c}	10.63^{c}	0.022^{c}	0.25c	0.16^{c}
$RL_{81}BL_{19}$	0.85^{a}	70.62^{a}	710.40^{a}	0.07^{a}	10.14^{a}	50.05^{a}	0.37^{ab}	10.97^{a}	30.68^{a}	0.065^{a}	0.633^{a}	0.52^{ab}
$RL_{70}BL_{21}GL_{9}$	0.63^{ab}	40.44^{abc}	480.96^{ab}	0.06^{ab}	0.71^{abc}	30.38^{ab}	0.42^{a}	10.30^{ab}	30.44^{ab}	0.062^{ab}	0.38^{b}	0.58^{a}
SEM	0.139	10.469	80.043	0.012	0.196	0.523	0.052	0.166	0.490	0.07	0.163	0.167
P	0.031	0.032	0.00	0.114	0.017	0.00	0.01	0.303	0.019	0.01	0.276	0.194

Values with different superscripts are significantly different at the $p = 0.05$ level by Tukey's multiple comparisons test. Light treatment abbreviation as shown in Figure 1.

root fresh weight at 27 DAS. No significant difference ($p>0.05$) between treatments at 27 and 37 DAS was also observed for root dry weight.

3.2 Effects of light treatments on chlorophyll and anthocyanin content

The estimated chlorophyll content (SPAD measurement) on purple-red pakchoi is shown in Figure 2. At each harvest day, the chlorophyll content was significantly higher ($p<0.05$) in plants treated with $RL_{81}BL_{19}$. Treatment with $RL_{70}BL_{21}GL_{9}$ light showed the SPAD reading was slightly lower than WL (control) at 37 DAS. However, there was no significant difference ($p>0.05$) between the SPAD reading. Figure 3 shows the result of the anthocyanin content in purple-red pakchoi as measured over time. The plants exposed under $RL_{81}BL_{19}$ light showed the highest anthocyanin levels (7.18 a.u, 27.02 a.u and 23.32 a.u, according to 17, 27 and 27 DAS, respectively) at each harvest day when compared to other treatments, with the highest anthocyanin level recorded on 27 DAS at 53.27 a.u.

4. Discussion

Light spectrum RL and BL can affect plant morphology (Johkan *et al.,* 2010). The RL and BL wavelengths trigger the highest photosynthetic performance rates, with RL being the most efficient. This leads to an impact on growth and development (McCree, 1971; Ouzounis *et al*., 2015). As reported in previous studies, the proportion of BL added to RL, from 2–15%, has a positive effect on the growth of lettuce (Hogewoning *et al*., 2010; Chen *et al*., 2016). Referring to this study, the positive impact can be seen for the percentage of BL at 19% and 21%. This study showed that the plant width and plant height at 27 DAS grown under $RL_{81}BL_{19}$ and $RL_{70}BL_{21}GL_{9}$ were almost the same as the plants exposed under WL at 37 DAS. Thus, it can be concluded that $RL_{81}BL_{19}$ and $RL_{70}BL_{21}GL_{9}$ light $treatment_{s}$ have induced the growth and morphology of purple-red pakchoi and sped up the harvest day compared to WL (control).

GL wavelength was slightly less efficient when compared to RL and RB wavelengths but still beneficial for photosynthesis (Johkan *et al*., 2012). GL was shown to improve the growth of lettuce when added to RL and BL mixture (Kim *et al*., 2004). Due to its deeper transmission through leaf tissue and canopy, it can be more efficient than RL in specific conditions (Terashima *et al*., 2009). Referring to the results in this experiment,

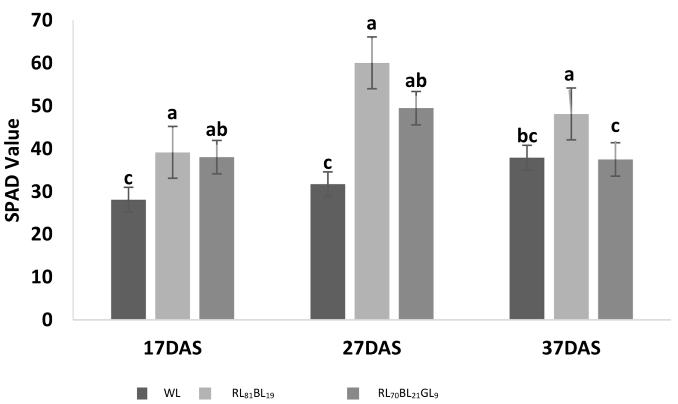

Figure 2. Estimated relative chlorophyll content in purple-red pakchoi plants over time for all light treatments. Bars with different notations are significantly different at $p = 0.05$ by Tukey's multiple comparisons tests. Light treatment abbreviations as shown in Figure 1.

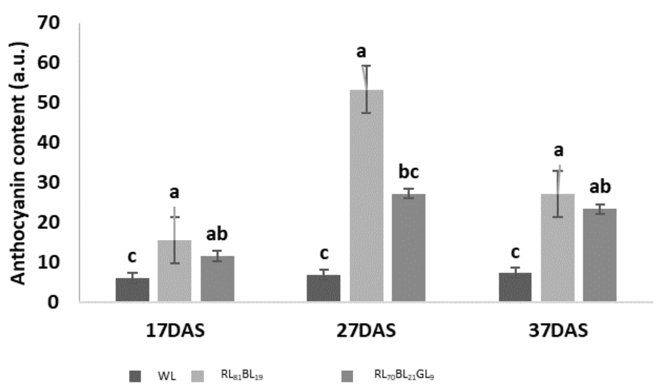

Figure 3. Estimated relative anthocyanin content in purple-red pakchoi plants over time for all light treatments. Bars with different notations are significantly different at $p = 0.05$ by Tukey's multiple comparisons tests. Light treatment abbreviations as shown in Figure 1.

https://doi.org/10.26656/fr.2017.6(S4).009

it seems that RL and BL treatments, supplemented with GL, have a slightly smaller size of plant height and width when compared to $RL_{81}BL_{19}$ light treatment. Plants under $RL_{81}BL_{19}$ exposure were found to respond to the narrow-band spectrum by increasing the leaf expansion, which is independent of the shade avoidance effect (Mickens *et al*., 2019). Referring to this research, leaf area expanded to the highest under $RL_{81}BL_{19}$ treatment even though there was no significant (p>0.05) when compared to plants treated with $RL_{70}BL_{21}GL_9$. Unlike lettuce crops, leaf expansion is often enhanced when GL wavelength is added to RL and BL or broad-spectrum lighting (Kim *et al*., 2004; Mickens *et al*., 2019)

The finding also showed that $RL_{81}BL_{19}$ treatment increased the chlorophyll content in most of the harvested purple-red pakchoi. Chlorophyll has maximum energy absorption within RL and BL wavelengths, with RL wavelength exposure having the highest quantum yield (Hogewoning *et al.*, 2012). This may indicate that spectra enriched with GL light have a tendency to reduce leaf chlorophyll levels in purple-red pakchoi. Chlorophyll concentrations can fluctuate based on the duration and light quality (Lefsrud *et al*., 2008). Anthocyanin is a crucial metabolite that protects plants against various biotic and abiotic stresses (Harborne and Williams, 2000). From the result, the $RL_{81}BL_{19}$ light exposure increased the anthocyanin level in purple-red pakchoi throughout the cultivation period. The $RL_{81}BL_{19}$ treatment improved the anthocyanin content of purple-red pakchoi (73 - 88%) when compared to WL (control).

On the other hand, in the plants under $RL_{70}BL_{21}GL_9$ treatment, the anthocyanin level was reduced to 47–74%, but still higher than the control. As shown in previous studies, BL light positively influenced the carotenoid and anthocyanin content of lettuce and tomato (Giliberto *et al*., 2005). GL light might be suppressing anthocyanin accumulation to allow deeper light penetration into the canopy (Hytönen *et al.*, 2018). This may explain the lower anthocyanin content in purple-red pakchoi, which responded to $RL_{70}BL_{21}GL_9$ treatment.

Nevertheless, it is highly likely that the different intensities between the light treatments also affected the plants' growth. Where WL had the lowest intensity, while $RL_{81}BL_{19}$ had the highest light intensity. Light intensity is one of the major factors that affect the photosynthesis process, plant development and nutritional values (Hartmann *et al*., 1981; Mickens *et al*., 2019). This experiment was carried out with lamp type constraints; errors such as intensity differences between lamp treatments will be avoided in future experiments.

4. Conclusion

The result of this study suggests that $RL_{81}BL_{19}$ light is the best light proportion for purple-red pakchoi production in indoor vertical farming. Both RL and BL wavelengths have a positive impact on the growth and accumulation of chlorophyll and anthocyanin content of purple-red pakchoi. The GL wavelength does not have a direct positive impact on the overall growth and accumulation of chlorophyll and anthocyanin content. This study supports that the selection of a specific light spectrum can be used to enhance crop morphology and nutritional attributes. It also demonstrated that there is no one light recipe that fits all crops. Each crop plant has its own optimal lighting recipe. Therefore, more studies need to carry out to investigate the effect of LED wavelength combinations on crop growth performance.

Acknowledgements

This research was funded by the MARDI Development Project under the 11th Malaysia Plan. We would like to thank the MARDI for the research facilities and financial support throughout the study.

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