AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Exogenous L-arginine and light-emitting diode light supplementation to enhance growth, quality and antioxidant activity in green perilla microgreens

Narongdech Patsadu[a], Parson Saradhuldhat[a], Jamnian Chompoo[b], Rahmat Budiarto[c], Supatida Abdullakasim[a,*]

[a] Department of Horticulture, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand

[b] Department of Agronomy, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand

[c] Department of Agronomy, Faculty of Agriculture, Universitas Padjadjaran, West Java 45363, Indonesia

Article Info

Article history:
Received 18 May 2023
Revised 27 September 2023
Accepted 29 September 2023
Available online 31 October 2023

Keywords:
DPPH,
Leaf area,
Light intensity,
Vitamin C

Abstract

Importance of the work: Green perilla is a medicinal plant rich in nutrients and bioactive compounds, especially at the microgreen stage. Enhancing the productivity and quality of green perilla microgreens can upgrade their product value for growers and provide increased variety for consumers regarding healthy products.

Objectives: To investigate the application of light-emitting diode (LED) illumination along with amino acid supplements, such as L-arginine to promote the growth, productivity and quality of green perilla microgreens.

Materials & Methods: The experimental design was a 4×4 factorial completely randomized design with two factors consisting of: 1) L-arginine concentrations (0 mM, 1.25 mM, 2.5 mM or 5 mM); and 2) LED lighting (white [W], red [R], blue [B] or red:blue 70:30 [70R:30B]). The plants were grown under a light intensity of 80 ± 5 μmol/m^2/s photosynthetic photon flux density, with an air temperature of 25°C and relative humidity of 65–70%.

Results: The 70R:30B LED lighting promoted not only the best growth (leaf fresh weight and leaf area) but also the best quality (nitrogen, crude protein, vitamin C, total phenolics and total flavonoids contents) of the microgreens. In addition, the white LEDs enhanced total chlorophyll, total carotenoids, nitrogen and crude protein. Furthermore, applying 5.0 mM of L-arginine improved the quality of the green perilla microgreens (total chlorophyll, carotenoids nitrogen and crude protein contents and 2,2-diphenyl-1-picrylhydrazyl scavenging activity). Since there was an interaction between the two factors, the combination of 5 mM L-arginine and the 70R:30B illumination significantly stimulated the plant height, stem fresh weight and total flavonoid contents of the microgreen product.

Main finding: Applying 70R:30B LEDs with 5 mM L-arginine was appropriate for green perilla microgreen production.

* Corresponding author.
E-mail address: fagrsds@ku.ac.th (S. Abdullakasim)

https://doi.org/10.34044/j.anres.2023.57.5.08

Introduction

Green perilla or perilla (*Perilla frutescens* [L.] Britt) is a medicinal plant belonging to the Lamiaceae or mint family (Ahmed, 2019). The leaves are used as a kitchen herb for cooking vegetables and decorating dishes and are eaten with meat and fish in numerous Asian countries, such as China, Japan and South Korea. The leaves have a unique aroma due to several phytochemical components. Currently, more than 270 phytochemical compounds have been isolated and reported in perilla seeds, stems and leaves (Ahmed, 2019), including: hydrophilic compounds, such as rosmarinic acid (a phenolic acid); apigenin and luteolin (flavonoids); volatile compounds, such as perillaldehyde, limonene, and perillene; and triterpene acids, such as tormentic acid, oleanolic acid (Ahmed, 2019). Perilla also has antioxidant, anti-inflammatory, anti-bacterial and anti-fungal activities (Qunqun, 2003; Huang et al., 2014; Tian et al., 2014; Ahmed, 2019). In addition, phenolic compounds, such as apigenin from the perilla leaf, produced antidepressant-like effects (Nakazawa et al., 2003). A recent vegetable consumption trend involves microgreens, of which nutrient-rich perilla is a good choice for consumers.

Microgreens are the seedlings of edible plants generally harvested when the first true leaves appear, usually 7–14 d after sowing. In several plants, the nutrients, phytochemical contents and antioxidant activity are higher in the microgreen stage than in the mature counterpart (Pinto et al., 2015; Waterland et al., 2017). In *Perilla frutescens* var. *crispa*, a high amount of volatile organic compounds was detected in the microgreen stage and reduced by approximately one-half at 4 wk in the mature stage (Dimita et al., 2022).

Light emitting diodes (LED) are widely applied in several plant production systems, especially in controlled-environment settings, to maximize growth, morphological uniformity and the accumulation of nutritional and phytochemical compounds. Red (600–700 nm) and blue (400–500 nm) light wavelengths are commonly used due to their effective absorption by plant photosynthetic pigments that stimulate photosynthesis (Nguyen and Oh, 2021). Red (R) light is involved with the phytochrome signaling mechanism; it is sensed by phytochrome receptors (PhyA and PhyB) and influences many mechanisms, such as germination, stem elongation, leaf expansion and flowering induction in plants (Li et al., 2011). In *Hypericum perforatum* L. cv. Topas, red light enhanced the fresh and dry weight, leaf area and number, plant height, flower number and diameter, as well as secondary metabolites, such as hypericin, pseudohypericin and hyperforin (Karimi et al., 2022). At age 6 wk, in the mature stage of *Perilla frutescens* var. *acuta* and *Perilla frutescens* var. *crispa*, R LEDs or a higher R ratio in combination treatments promoted the best shoot and root fresh weight, total leaf area, antioxidant capacity and rosmarinic acid content compared to other light spectra, such as blue (B) LEDs (Nguyen and Oh, 2021).

B LEDs stimulate the opening of stomata and increase the ratio of chlorophyll a-to-b (Hogewoning et al., 2010). Increasing the B light dose up to 50% resulted in the maximum photosynthetic capacity compared to the lower B light ratio in *Cucumis sativus* (Hogewoning et al., 2010). The R+B combination enhances growth characteristics and secondary metabolite contents more so than monochrome R and monochrome B light sources in many crops, since the B and R light are major wavelengths sensed by plant pigments and phytochromes (Meas et al., 2020). However, in *Ocimum basilicum* L. microgreens, predominantly B illumination (R1:B2) stimulated greater fresh and dry masses and improved some bioactive compound accumulation, such as caffeic acid and rosmarinic acid, compared to 2R:1B or 1R:1B (Lobiuc et al., 2017). Therefore, plant species may have diverse responses to LED wavelengths and ratios.

Arginine is a proteinogenic amino acid that has the highest nitrogen-to-carbon ratio and is an essential amino acid for protein synthesis, a precursor for proline, polyamines and nitric oxide (NO), and is involved in many plant metabolisms (Winter et al., 2015). Exogenous application of arginine allows the direct uptake of organic nitrogen resources by plants, enhancing nitrogen accumulation (Näsholm et al., 2009) and increasing yield and quality (Wang et al., 2021). In tomatoes, foliar applications of arginine improved yield and quality components, such as lycopene, vitamin C, soluble sugar contents and nitrogen accumulation (Wang et al., 2021). Additionally, it can protect tomatoes from transient heat stress, probably due to decreasing the hydrogen peroxide level to avoid oxidative imbalance with an increased concentration of arginine application (Conceição et al., 2021). In ora-pro-nobis plants, applying L-arginine enhanced their net photosynthetic rate, leaf area and fresh and dry masses and increased secondary metabolites, such as carotenoids and crude protein, in the leaves (Freitas et al., 2022).

To date, there have been few published reports regarding the influence of LEDs on green perilla microgreens. In particular, the application of L-arginine in microgreens has yet to be investigated thoroughly. Therefore, the current study used both the LED type and L-arginine factors to clarify their effects on the growth and quality of perilla microgreens. The findings should benefit microgreen production and may be applicable to other crops.

Materials and Methods

Plant material and growth condition

Perilla seeds (Chua Yong Seng Seed Co., Ltd) at 0.7 g per pot were grown in plastic pots (dimensions 8 cm × 6 cm × 8 cm). Peat moss was used as the growing substrate. The pots were grown under different LED spectra consisting of white, R, B and R+B 70:30 (70R:30B). L-arginine solution was added at 30 mL per pot on days 7 and 14 after seed sowing. The experimental design was 4×4 factorial completely randomized design, including two factors: 1) type of light spectrum —white, R, B, and R+B 70R: 30B– as shown in Figs. 1 and 2) concentration of L-arginine— 0 mM, 1.25 mM, 2.5 mM and 5.0 mM. Each treatment was applied to four replications. Growth conditions were maintained under light intensity at 80 ± 5 µmol/m^2/s, light for 12 hr/d, a temperature of 25±2°C and relative humidity of 80–85%.

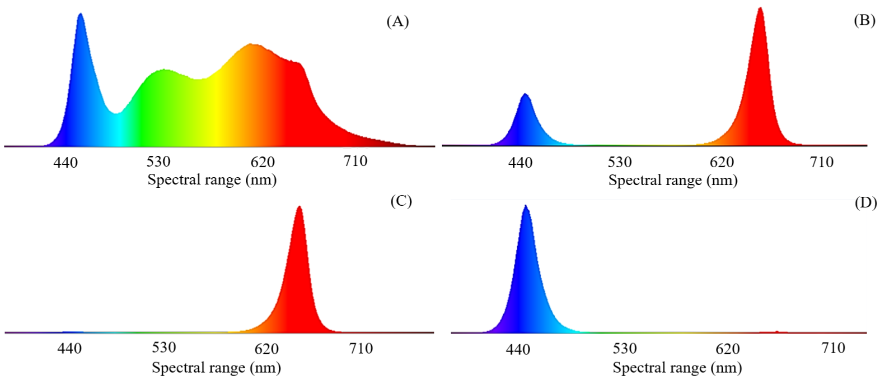

Fig. 1 Spectral ranges used in the study: (A) white (430–780 nm); (B) 70 red:30Bblue; (C) red light (646 nm peak); (D) blue light (451 nm peak)

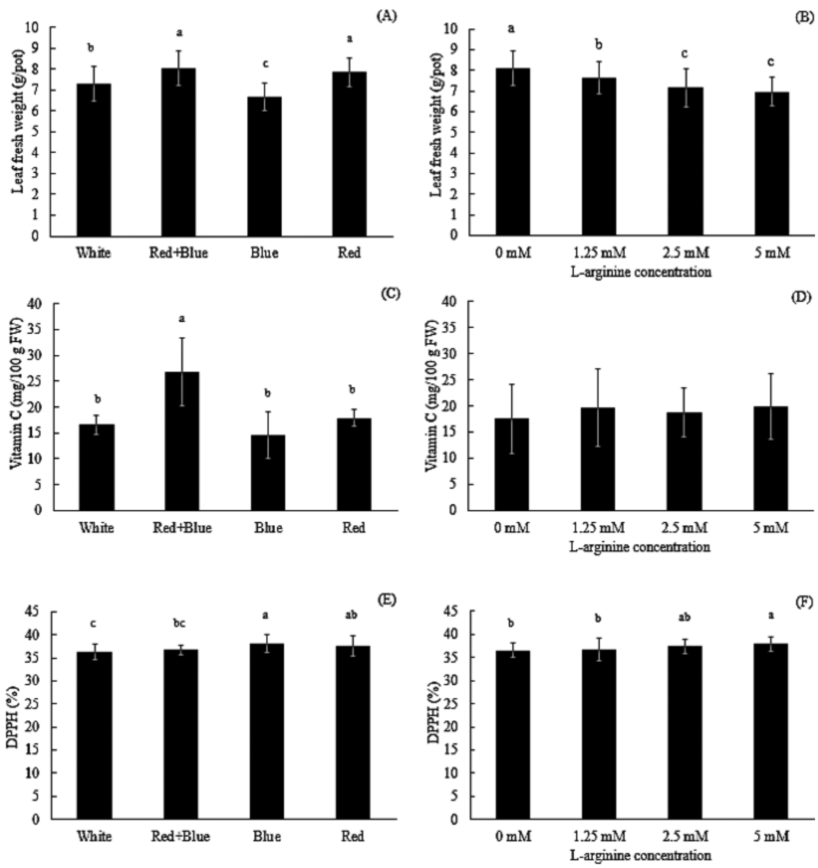

Fig. 2 Leaf fresh weight, vitamin C and 2,2-diphenyl-1-picrylhydrazyl (DPPH) activity of perilla microgreens grown under controlled conditions, where mean (± SD) calculated from four replicates for each treatment and bars with different lowercase letters are significantly ($p < 0.05$) different.

Measurement of growth performance

At 21 d after seed sowing, the microgreens in each pot were cut at 0.5 cm above the growing substrate level. The small microgreens (four replications per pot) were measured for plant height from the cut edge to the top shoot. Then, all microgreens per a pot were separated into their leaf and stem parts for fresh weight measurement. Next, 30 shoots per pot were randomly selected and measured for total leaf area using a leaf area meter (LI-3000A; Li-Cor; Lincoln, NE, USA).

Determination of chlorophyll a, b and total carotenoids contents

The amounts of chlorophyll a, b and total carotenoids were determined using the spectrophotometric method according to Holden (1976), with slight modification. A perilla microgreen frozen sample (1 g) was homogenized with 9 mL of 80% acetone for 1 min using a homogenizer (PolyTron model PT 2100; Kinematica AG Inc.; Lucerne, Switzerland). After that, it was transferred to an Erlenmeyer flask and shaken using an orbital shaker (model GFL 3017; Burgwedel, Germany) for 24 hr at 5°C or until the precipitant became colorless. The sample was centrifuged (Centurion Scientific; model 1010D; West Sussex, UK) at 10,000 revolutions per minute (rpm) for 15 minutes at room temperature. The supernatant was measured using a spectrophotometer (GENESYS10; Thermo Scientific; Texas, TX, USA) at wavelengths of 470 nm, 645 nm and 662 nm. The calculations for chlorophyll a, b, chlorophyll (a + b) and total carotenoids contents were based on Equations 1–4, respectively:

$$\text{Chlorophyll a (Chl a)} = 11.75\ A_{662} - 2.350\ A_{645} \quad (1)$$

$$\text{Chlorophyll b (Chl b)} = 18.61\ A_{645} - 3.960\ A_{662} \quad (2)$$

$$\text{Chlorophyll (a + b)} = \text{Chl a} + \text{Chl b} \quad (3)$$

$$\text{Total carotenoids} = (1{,}000\ A_{470} - 2.270\ \text{Chl a} - 81.4\ \text{Chl b}) / 227 \quad (4)$$

where A_{xxx} indicates the wavelength at xxx nm.

Total vitamin C

The ascorbic acid content was estimated using the 2,6-dichlorophenolindophenol titration method (Association of Official Analytical Chemists, 1990). A sample (10 g) of fresh perilla microgreens was homogenized with 20 mL of acetic acid and centrifuged at 12,000 rpm for 2 min using the centrifuge. Supernatant (2 mL) was added to the extraction buffer containing 0.2 mL of 2,6-dichlorophenolindophenol (0.2%). The reaction mixture was measured at 540 nm. The concentration of ascorbic acid was calculated using a regression curve of different rates (0–50 μg/mL) of pure ascorbic acid. The result was expressed as milligrams per gram fresh weight.

Preparation of extracts for determination of total flavonoids, total phenolics and 2,2-diphenyl-1-picrylhydrazyl scavenging

The leaves and stem of the perilla microgreens were dried at 70°C for 72 hr using an oven (Contherm; model Thermotec 2000 series; Lower Hutt, New Zealand) and then ground in a blender. Dried powder samples (each 1 g) were each macerated with 10 mL of ethanol (80%) and incubated at room temperature (25°C). After 24 hr, the extracts were passed through Whatman® paper filter No. 1. The extracts were diluted with 80% ethanol to final concentrations of 1,000 μg/mL for determining total flavonoids, total phenolics and DPPH scavenging activity.

Determination of total flavonoids

The total flavonoid content was determined according to colorimetric assay, modified from Djeridane et al. (2006). The perilla microgreen extracts (each 100 μL) at a concentration of 1,000 μg/mL were each mixed with 100 μL of aluminum chloride (2% weight per volume. w/v) in a 96-well plate and incubated at ambient temperature for 15 min. Then, the solution was measured at an absorbance of 430 nm using a microplate reader (Multiskan Go; Thermo Scientific; Vantaa, Finland). The total flavonoid content in each extract was compared with the rutin standard curve, where Y= 0.007x - 0.003 (coefficient of determination, $R^2 = 0.999$). The result was expressed as milligrams of rutin equivalents (RUE) per 1 mg of extract.

Determination of total phenolics

The total phenolic content was determined using the method from Siddhuraju and Becker (2003), with some modifications. The extract samples (each 20 μL) were each mixed with 50 μL of distilled water and 50 μL of Folin-phenol reagent (reagent-to-water ratio = 1:1) in a 96-well plate and then added with 80 μL of sodium carbonate (7.5% w/v). The solution was incubated at ambient temperature for 30 min and measured at an absorbance of 725 nm using the microplate reader. Gallic acid (0.001–0.01 mg/mL) was used as a standard. The total phenolic content in each extract was compared with the standard curve, where Y=0.0079x + 0.055 (R^2=0.997). The result was expressed as milligrams of gallic acid equivalents (GAE) per 1 mg of solid crude extract.

Determination of 2,2-diphenyl-1-picrylhydrazyl scavenging activity

The total antioxidant capacity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, with a slight modification (Boskou et al., 2006) and mixing 100 µL of the prepared green perilla microgreen extract with 40 µL of DPPH solution (Sigma-Aldrich, Inc.; Burlington, MA, USA) at a concentration of 0.5 mM and 100 µL of sodium acetate buffer (0.1 M, pH 5.5). Then, the solution was kept in the dark at room temperature (25±2°C) for 30 min and measured at an absorbance of 517 nm using the microplate reader. Tert-butylhydroxytoluene (BHT; Sigma-Aldrich, Inc.; Burlington, MA, USA) was used as a positive control for comparing antioxidant capacity. The radical scavenging percentage was calculated using Radical scavenging (%) = $[(OD_{control} - OD_{sample}) / OD_{control}] \times 100$, where OD is the optical density in the presence or absence of the sample.

Nitrogen content and crude protein

Perilla microgreen samples (each 10 g) were dried at 70°C for 72 hr and then were finely ground. After that, 0.1 g of each sample was used for nitrogen determination with a protein/nitrogen determinator (model FP–528; LECO; San diego, CA, USA). In general, crude protein contains about 16% nitrogen. Therefore, the crude protein (%) was derived from the amount of N (%) multiplied by 6.25.

Statistical analysis

Statistical analyses were performed using 2-way analysis of variance, with mean differences evaluated using the least significant difference (LSD) test at the $p < 0.05$ significance level

Results

Growth performance of perilla microgreens

The influence of the light wavelengths and L-arginine application on the growth performance of the perilla microgreens is shown in Table 1 and Fig. 2. The presented data revealed that the leaf area of the perilla microgreens grown under 70R:30B was the highest and significantly different from the monochromatic R or B treatments. However, the monochromatic R light promoted the best stem fresh weight, while the monochromatic B light produced the greatest height of the perilla microgreens. The leaf fresh weights of the perilla microgreens grown using 70R:30B and R were the highest and significantly different from the white or B treatments (Fig. 2).

Table 1 Mean comparison of light spectra × L-arginine interaction effects on growth performance and antioxidant-related parameters of perilla microgreens

Light spectrum	L-arginine (mM)	Leaf area (cm²/pot)	Stem fresh weight (g/pot)	Plant height (cm)	Total flavonoids (mg RUE/g extract)	Total phenolics (GAE/g extract)
White	0	10.98±1.32^{cd}	5.16±0.68^{cdef}	8.25±0.67^{fgh}	13.25±0.71^{cdef}	1.39±0.31^{de}
	1.25	11.37±0.87^{bc}	5.80±0.59^{abcd}	8.85±0.40^{ef}	13.89±2.48^{bcde}	1.69±0.39^{cde}
	2.50	13.66±1.90^{a}	4.84±0.49^{defg}	7.90±0.30^{gh}	14.08±2.57^{bcde}	1.21±0.17^{e}
	5.00	14.21±2.47^{a}	5.24±0.41^{cdef}	8.13±0.17^{fgh}	12.81±2.02^{def}	1.51±0.17^{de}
Red:Blue70:30	0	14.50±1.00^{a}	3.93±0.33^{gh}	7.06±0.17^{i}	14.43±3.04^{bcd}	1.98±0.37^{bcde}
	1.25	13.11±2.75^{abc}	3.85±0.33^{gh}	7.57±0.20^{hi}	17.70±1.88^{a}	3.01±1.21^{a}
	2.50	13.44±1.34^{ab}	4.44±0.55^{fgh}	7.47±0.27^{hi}	12.51±0.90^{defg}	2.45±0.83^{ab}
	5.00	13.96±0.74^{a}	4.65±0.09^{efgh}	7.72±0.28^{hi}	16.12±0.78^{ab}	2.35±0.38^{abc}
Blue	0	8.75±0.90^{ef}	6.30±1.04^{ab}	9.68±1.00^{cd}	13.82±1.46^{bcde}	2.84±0.14^{a}
	1.25	12.52±0.98^{abc}	6.75±0.70^{a}	11.43±0.66^{a}	10.97±0.86^{fg}	1.45±0.17^{de}
	2.50	11.00±0.50^{cd}	5.25±0.48^{cdef}	10.70±0.43^{ab}	9.96±0.80^{g}	1.63±0.07^{cde}
	5.00	10.46±1.55^{de}	5.48±0.84^{bcde}	10.40±0.98^{bc}	10.78±1.71^{fg}	1.56±0.35^{de}
Red	0	6.17±0.50^{g}	6.08±0.54^{abc}	8.68±0.30^{ef}	11.41±1.04^{efg}	1.62±0.49^{cde}
	1.25	7.80±1.04^{fg}	6.30±1.01^{ab}	9.63±0.53^{cd}	11.68±0.74^{defg}	1.75±0.09^{bcde}
	2.50	6.46±0.73^{g}	6.72±0.48^{a}	9.99±0.49^{bcd}	15.54±1.67^{abc}	2.01±0.35^{bcd}
	5.00	6.71±1.37^{fg}	5.89±0.45^{abc}	9.32±0.29^{de}	11.88±1.63^{defg}	1.79±0.38^{bcde}

RUE = rutin equivalents; GAE = gallic acid equivalents

Values are means±SD ($n = 4$), where different lowercase superscripts in a column indicate significant differences between treatments (LSD test at $p < 0.01$).

Ther light wavelength and L-arginine application interacted with growth parameters, such as leaf area, stem fresh weight and plant height (Table 1). The application of L-arginine (1.25 mM) with B light significantly improved the stem fresh weight, height and leaf area of the microgreens (Table 1). Similarly, L-arginine (5.0 mM) in combination with white light also increased the leaf area. In addition, the combination of 5 mM L-arginine and 70R:30B greatly stimulated the stem fresh weight and plant height compared to the non L-arginine application. However, the other combinations of light wavelengths and L-arginine application had no significant influence on the growth parameters (Table 1).

Antioxidant capacity and related parameters

The 70R:30B LEDs promoted the significantly highest accumulation of vitamin C, total flavonoids and total phenolic (Table 1 and Fig. 2). In addition, the B treatments promoted the highest percentage of DPPH scavenging activity, followed by the R treatments. Application of L-arginine (1.25–5.0 mM) did not significantly affect the vitamin C production (Fig. 2). However, the % DPPH scavenging activity increased, consistent with the increasing the L-arginine concentration, with the 5.0 mM L-arginine concentration promoting the highest % DPPH scavenging activity (Fig. 2). There was an interaction between the light wavelength and L-arginine application on the total flavonoids, and total phenolic contents. The combination of 70R:30B light and L-arginine at 1.25 mM or 5.0 mM promoted the highest accumulation of total flavonoid contents (Table 1). However, L-arginine (1.25–5 mM) combined with the B light treatments had a negative impact on the total flavonoid and total phenolic contents (Table 1).

Photosynthetic-related pigments

Total chlorophyll (a+b) levels were the highest in the white LED treatment, followed by the 70R:30B treatment. The monochromatic R or B light treatments had the lowest amounts of total chlorophyll (Fig. 3). Similarly, the white LED promoted the highest yields of total carotenoids followed by the 70R:30B treatment, while the monochromatic B or R treatments had the lowest total carotenoid contents. Application of L-arginine at 5 mM promoted the highest total chlorophyll and total carotenoid contents compared to the other treatments (Fig. 3). There was no interaction between the light wavelength and L-arginine application on the accumulation of photosynthetic-related pigments in perilla microgreens.

Nitrogen content and crude protein

The white and 70R:30B light treatment produced higher nitrogen and crude protein accumulation (6.47–6.51% dry weight and 40.46–40.60% dry weight, respectively). At the same time, the sole R or B wavelengths produced lower nitrogen and crude protein accumulation (6.03–6.16% dry weight and 37.46–38.52% dry weight respectively),

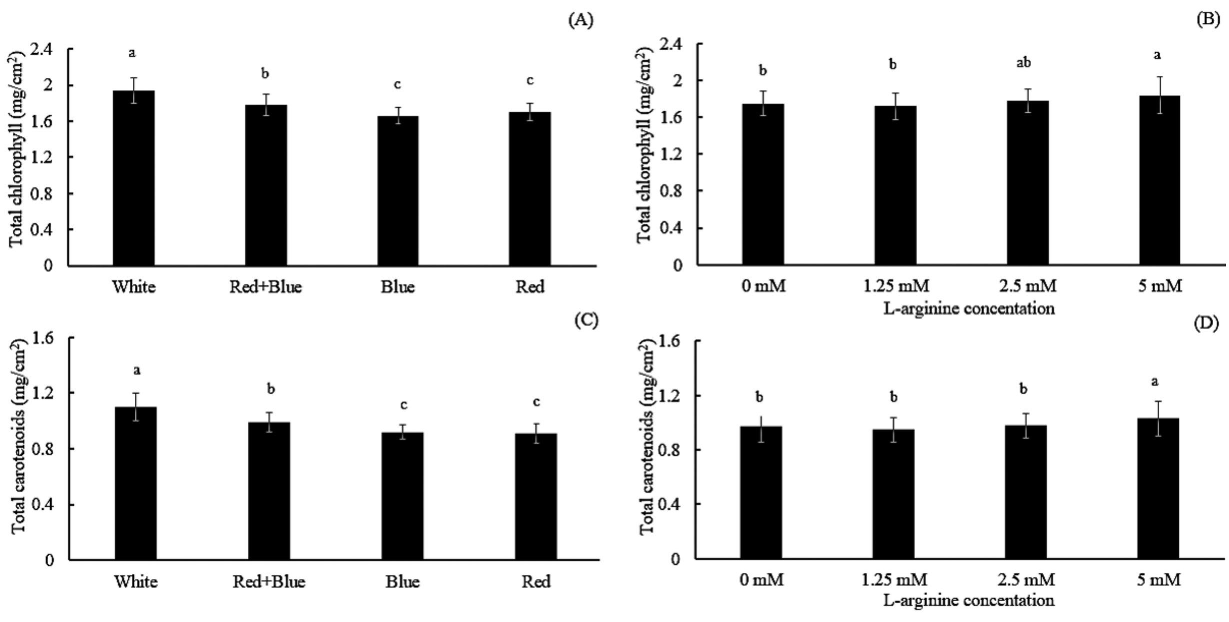

Fig. 3 Total chlorophyll and carotenoids of perilla microgreens grown under controlled conditions, where mean (± SD) calculated from four replicates for each treatment and bars with different lowercase letters are significantly ($p < 0.05$) different.

as shown in Fig. 4. The application of L-arginine significantly increased the nitrogen and crude protein contents. The levels of nitrogen and crude protein accumulation gradually increased with increasing L-arginine concentration. The highest nitrogen and crude protein contents were detected in the 5 mM L-arginine treatments (6.61% dry weight and 41.34% dry weight, respectively), while same parameters for the non-treated control were only 6.03% dry weight and 36.83% dry weight, respectively (Fig. 4).

Discussion

R and B wavelengths have a powerful influence on plant growth, development, and secondary metabolism because the spectral region of 400–700 nm is necessary for photosynthetic reactions covering the R (600–700 nm) and B (400–499 nm) regions (Rabara et al., 2017). Chlorophyll pigments can potentially absorb both R and B wavelengths, making up to 80–95% of the total light absorbed (Terashima et al., 2009). R light has a higher quantum yield than B light, resulting in R light pigments having greater light absorption efficiency (McCree, 1972, Hogewoning et al., 2010). In *Cucumis sativus*, only 7% B light (combined with 93% R light) was adequate to avoid overt dysfunctional photosynthesis (Hogewoning et al., 2010). Based on the overall results of the current study, the 70R:30B combination promoted the best growth and quality of perilla microgreens, based on their leaf fresh weight, leaf area, percentage of nitrogen and crude protein contents, vitamin C, total flavonoids and total phenolics contents compared to the white or monochromatic R or B LEDs lights. R light was predominant in promoting leaf biomass and number of leaves compared to B light for *Hypericum perforatum* L. cv. Topas (Rabara et al., 2017). This involves the role of R light in stimulating phytochromes to regulate plant development (Tripathi et al., 2019). R light can induce cell division, as shown by evidence that R light enhanced mRNA synthesis to produce nucleolin, before any cell proliferation during the de-etiolation of pea, where the nucleolin is functionally involved in ribosome biosynthesis (Reichler et al., 2001). In amaranth microgreens, the 70R:30B promoted the best fresh weight and pigment compounds compared to monochromatic R or B lights; thus, a high amount of total chlorophyll may support high growth performance of this microgreen (Meas et al., 2020). In *Digitalis purpurea* L. grown under a closed type plant factory, R:B (8:2) light promoted the best leaf area and number of leaves compared to R:B (1:1 or 2:8) or fluorescence and monochromatic R or B lights (Verma et al., 2018).

In the current study, the white light had the highest chlorophyll content per square centimeter of leaf area, followed by the 70R:30B treatment. However, the 70R:30B treatment had a greater leaf area that facilitated photosynthesis, resulting in a significant biomass increase. In addition, the R:B combination enhanced the maximum number of stomata in *Hypericum perforatum* L. cv. Topas, compared to monochromatic R or B LEDs (Karimi et al., 2022), which was probably related to the increased photosynthetic efficiency in the 70R:30B treatment for the green perilla. Consistent with the total chlorophyll contents, nitrogen and crude protein accumulation was relatively high in the white light treatment. However, there were no significant differences between the white light and 70R:30B treatments. The total chlorophyll content is related to nitrogen accumulation, since it is necessary for the nitrogen pathway (Wang et al., 2014). Furthermore, a high R:B could enhance better nitrogen absorption and translation to soluble protein by stimulating the transcription levels of nitrogen metabolism-related enzymes compared to the monochrome R or B light (Wang et al., 2017).

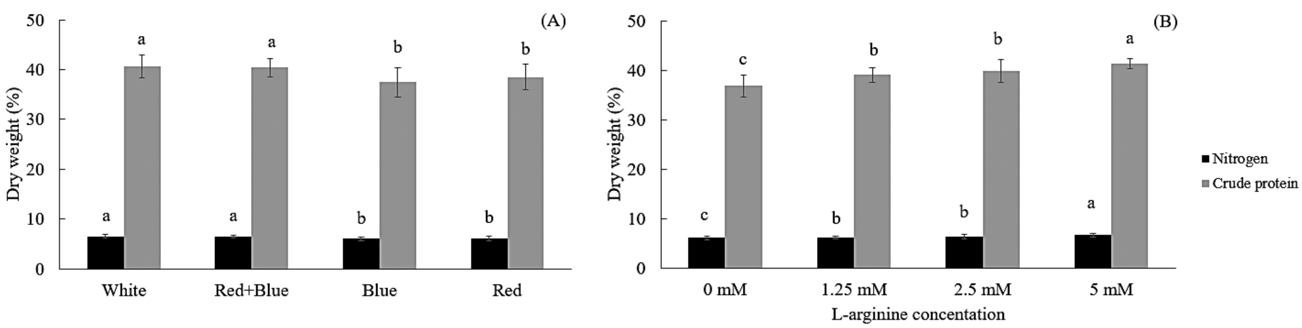

Fig. 4 Nitrogen and protein contents of perilla microgreen grown under different light-emitting diode and L-arginine treatments, where mean (± SD) calculated from four replicates for each treatment and bars with different lowercase letters are significantly ($p < 0.05$) different.

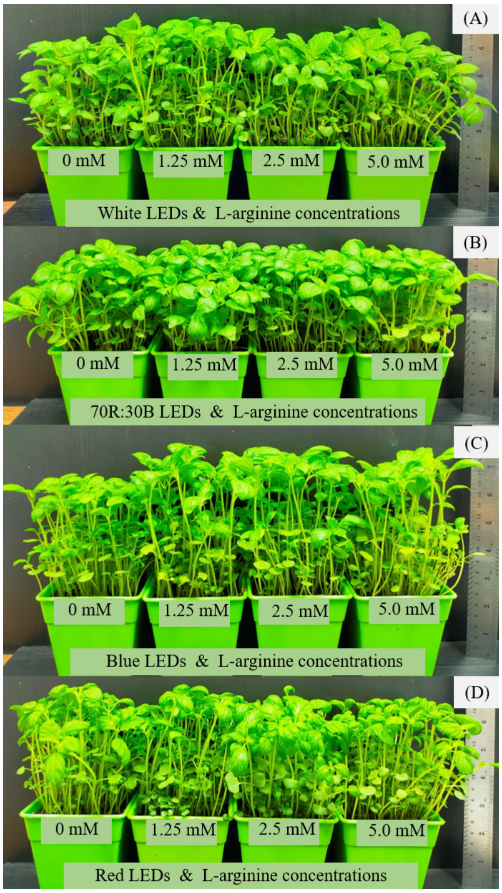

Fig. 5 Perilla microgreens grown under different light-emitting diode (LED) treatments and control conditions at 21 d after sowing: (A) white; (B) 70R:30B; (C) blue; (D) red, where largest leaf area for 70R:30B reflects consumer-preferred characteristics for perilla microgreens

Vitamin C, total phenolics and total flavonoids have antioxidant properties that can protect plants from abiotic stress by reducing reactive oxygen species (ROS) levels (Aryal et al., 2019; Xiao et al., 2021). A flavonoid is a group of phenolics with potent antioxidant properties. In the current study, the 70R:30B treatment produced the best vitamin C, total phenolics and total flavonoid accumulation, though responses have been reported to vary among plant species (Meas et al., 2020). The combination of R and B light in the appropriate proportion enhanced the levels of total phenolics, total flavonoids and antioxidant activity, especially with a higher proportion of B light. The irradiation of B light likely stimulates the activity of phenylalanine ammonia-lyase (PAL), a key enzyme in the phenylpropanoid pathway for pigmentation (Heo et al., 2012; Son and Oh, 2013). For example, in lettuce, the mix of R:B (1:1) greatly induced the PAL enzyme activity and stimulated a high amount of protein accumulation compared to monochromatic R or B lights (Heo et al., 2012).

In the current study, the monochromatic B light significantly promoted the height of green perilla microgreens compared to the other treatments. This was consistent with the results for some bedding plants (petunia, calibrachoa, geranium and marigold) where B light (at 50 μmol/m^2/s or 100 μmol/m^2/s) promoted elongation growth, where this evidence was related to lower phytochrome activity, as a kind of shade-avoidance response in plants (Kong et al., 2018). In addition, a low light intensity, especially with the B light treatment used in the current study (80 μmol m/^{2}/s), probably caused low light intensity stress to the microgreens, since they responded by accumulating a high level of antioxidants (evaluated based on DPPH scavenging assay). Similar results were reported for B light-grown callus cultures of *Rhodiola imbricata* (Kapoor et al., 2018).

Application of L-arginine, especially at the high concentration (5 mM), significantly promoted the leaf area, the antioxidation activity and the contents of total chlorophyll, carotenoids, nitrogen and crude protein of the green perilla microgreens (Table 1, Figs. 3 and 4). Arginine contains a high nitrogen-to-carbon ratio and acts as a form of nitrogen storage form in plants (Winter et al., 2015). Therefore, the exogenous L-arginine application enhanced the high level of nitrogen and crude protein accumulation in the green perilla microgreens. Furthermore, exogenous L-arginine application increased the photosynthetic rate and increased biomass and the leaf area (Freitas et al., 2022). Increasing the leaf area facilitated efficient photo-assimilation, resulting in plant biomass accumulation. In addition, arginine contributes to the stress response in plants, since it is a precursor or mediator in several biosynthetic pathways regarding plant stress, such as proline and polyamine biosynthesis (Winter et al., 2015). The increased DPPH antioxidant activity in green perilla microgreens through the application of L-arginine was probably due to the high amount of precursor or mediator for the antioxidant-related pathway. Where a plant is under stress, an L-arginine application will help protect the plant by reducing the ROS level, with the antioxidant level also decreasing.

There were interactions between the light wavelength and the level of L-arginine application for some growth parameters, such as stem fresh weight, leaf area, plant height, total phenolic and flavonoids; therefore, the combination of 70R:30B light with L-arginine at an appropriate concentration (5 mM) could enhance the growth and quality of the green perilla microgreens.

Conclusion

The combination of 70R:30B LEDs had high potential to improve the growth and quality of green perilla microgreens compared to monochromatic R or B LEDs. The significant increase in leaf area under the 70R:30B treatment resulted in excellent consumer-preferred characteristics for the microgreen product. In addition, the 70R:30B LEDs promoted antioxidant activity and the levels of vitamin C, total phenolics and total flavonoids. Exogenous application of L-arginine at 5 mM promoted the leaf area and the nitrogen and crude protein contents in the microgreens. The two factors investigated (LED light and L-arginine supplementation) significantly interacted regarding the leaf area and some antioxidant-related parameters. These findings should assist growers to modify their current regimes to increase the yield and quality of their microgreens in the near future by determining the precise factors for perilla microgreen production, such as using a combination of 70R:30B LEDs and 5 mM L-arginine to obtain better yields and increase the nutrient level and antioxidant property of the marketed products.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

Advance Electric & Electronic Company Limited provided the LED lamps used in this study.

References

Ahmed, H.M. 2019. Ethnomedicinal, phytochemical and pharmacological Investigations of *Perilla frutescens* (L.) Britt. *Molecules 24*: 102. doi.org/10.3390/molecules24010102

Aryal, S., Baniya, M.K., Danekhu, K., Kunwar, P., Gurung, R., Koirala, N. 2019. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from western Nepal. Plants 8: 96. doi: 10.3390/plants8040096

Association of Official Analytical Chemists. 1990. Vitamin C (ascorbic acid) in vitamin preparations and juices. Official Methods of Analysis, 15th ed. Association of Office Analytical Chemists. Arlington, VA, USA, pp. 1058–1059.

Boskou, G., Salta, F.N., Chrysostomou, S., Mylona, A., Chiou, A., Andrikopoulos, N.K. 2006. Antioxidant capacity and phenolic profile of table olives from the Greek market. Food Chem. 94: 558–564. doi.org/10.1016/j.foodchem.2004.12.005

Conceição, V.J., Mello, S.C., Carvalho, M.E.A, Gaziola, S.A., Azevedo, R.A. 2021. Exogenous arginine modulates leaf antioxidant enzymes and hydrogen peroxide content in tomato plants under transient heat stresses. Bragantia 80: e2621. doi.org/10.1590/1678-4499.20200493

Dimita, R., Allah, S.M., Luvisi, A., Greco, D., De Bellis, L., Accogli, R., Mininni, C., Negro, C. 2022. Volatile compounds and total phenolic content of *Perilla frutescens* at microgreens and mature stages. Horticulturae 8: 71. doi.org/10.3390/horticulturae8010071

Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P., Vidal, N. 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolics. Food Chem. 97: 654–660. doi.org/10.1016/j.foodchem.2005.04.028

Freitas, I.S., Trennepohl, B.I., Acioly, T.M.S., Conceição, V.J., Mello, S.C., Dourado Neto, D., Kluge. R.A., Azevedo, R.A. 2022. Exogenous application of L-arginine improves protein content and increases yield of *Pereskia aculeata* Mill. grown in soilless media container. Horticulturae 8: 142. doi.org/10.3390/horticulturae8020142

Heo, J.W., Kang, D.H., Bang, H.S., Hong, S.G., Chun, C., Kang, K.K. 2012. Early growth, pigmentation, protein content, and phenylalanine ammonia-lyase activity of red curled lettuces grown under different lighting conditions. Kor. J. Hort. Sci. Technol. 30: 6–12. dx.doi.org/10.7235/hort.2012.11118

Hogewoning, S.W., Trouwborst, G., Maljaars, H., Poorter, H., van Ieperen, W., Harbinson, J. 2010. Blue light dose-responses of leaf photosynthesis, morphology, and chemical composition of *Cucumis sativus* grown under different combinations of red and blue light. J. Exp. Bot. 61: 3107–3117. doi.org/10.1093/jxb/erq132

Huang, B.P., Lin, C.H., Chen, Y.C., Kao, S.H. 2014. Anti-inflammatory effects of *Perilla frutescens* leaf extract on lipopolysaccharide-stimulated RAW264. 7 cells. Mol. Med. Rep. 10: 1077–1083. doi.org/10.3892/mmr.2014.2298

Kapoor, S., Raghuvanshi, R., Bhardwaj, P., Sood, H., Saxena, S., Chaurasia, O.P. 2018. Influence of light quality on growth, secondary metabolites production and antioxidant activity in callus culture of *Rhodiola imbricata* Edgew. J. Photochem. Photobiol. B 183: 258–265. doi.org/10.1016/j.jphotobiol.2018.04.018

Karimi, M., Ahmadi, N., Ebrahimi, M. 2022. Red LED light promotes biomass, flowering and secondary metabolites accumulation in hydroponically grown *Hypericum perforatum* L. (cv. Topas). Ind. Crops Prod. 175: 114239. doi.org/10.1016/j.indcrop.2021.114239

Kong, Y., Stasiak, M., Dixon, M.A., Zheng, Y. 2018. Blue light associated with low phytochrome activity can promote elongation growth as shade-avoidance response: A comparison with red light in four bedding plant species. Environ. Exp. Bot. 155: 345–359. doi.org/10.1016/j.envexpbot.2018.07.021

Li, J., Li, G., Wang, H., Deng, X.W. 2011. Phytochrome signaling mechanisms. Arabidopsis Book 9: e0148. doi.org/10.1199/tab.0148

Lobiuc, A., Vasilache, V., Pintilie, O., Stoleru, T., Burducea, M., Oroian, M., Zamfirache, M.M. 2017. Blue and red LED illumination improves growth and bioactive compounds contents in acyanic and cyanic *Ocimum basilicum* L. microgreens. Molecules 30: 22: 2111. doi.org/10.3390/molecules22122111

McCree, K.J. 1972. The action spectrum, absorptance and quantum yield of photosynthesis in crop plants. Agr. Meteorol. 9: 191–216. doi.org/10.1016/0002-1571(71)90022-7

Meas, S., Luengwilai, K., Thongket, T. 2020. Enhancing growth and phytochemicals of two amaranth microgreens by LEDs light irradiation. Sci. Hort. 265: 109204. doi.org/10.1016/j.scienta.2020.109204

Nakazawa, T., Yasuda, T., Ueda, J., Ohsawa, K. 2003. Antidepressant-like effects of apigenin and 2, 4, 5-trimethoxycinnamic acid from *Perilla frutescens* in the forced swimming test. Biol. Pharm. Bull. 26: 474–480. doi.org/10.1248/bpb.26.474

Näsholm, T., Kielland, K., Ganeteg, U. 2009. Uptake of organic nitrogen by plants. New. Phytol. 182: 31–48. doi.org/10.1111/j.1469-8137.2008.02751.x

Nguyen, T.K.L., Oh, M.M. 2021. Physiological and biochemical responses of green and red perilla to LED-based light. J. Sci. Food. Agric. 101: 240–252. doi.org/10.1002/jsfa.10636

Pinto, E., Almeida, A.A., Aguiar, A.A., Ferreira, I.M.P.L.V.O. 2015. Comparison between the mineral profile and nitrate content of microgreens and mature lettuces. J. Food Compos. Anal. 37: 38–43. doi.org/10.1016/j.jfca.2014.06.018

Qunqun, G. 2003. Antibacterial activity of *Perilla frutescens* leaf essential oil. Sci. Technol. Food. Ind. 9: 019.

Rabara, R.C., Behrman, G., Timbol, T., Rushton, P.J. 2017. Effect of spectral quality of monochromatic LED lights on the growth of artichoke seedlings. Front. Plant Sci. 8: 190. doi.org/10.3389/fpls.2017.00190

Reichler, S.A., Balk, J., Brown, M.E., Woodruff, K., Clark, G.B., Roux, S.J. 2001. Light differentially regulates cell division and the mRNA abundance of pea nucleolin during de-etiolation. Plant Physiol. 125: 339–350. doi.org/10.1104/pp.125.1.339

Siddhuraju, P., Becker, K. 2003. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. J. Agric. Food Chem. 51: 2144–2155. doi.org/10.1021/jf020444+

Son, K.H., Oh, M.M. 2013. Leaf shape, growth, and antioxidant phenolic compounds of two lettuce cultivars grown under various combinations of blue and red light-emitting diodes. Hort. Sci. 48: 988–995. doi.org/10.21273/HORTSCI.48.8.988

Terashima, I., Fujita, T., Inoue, T., Chow, W.S., Oguchi, R. 2009. Green light drives leaf photosynthesis more efficiently than red light in strong white light: Revisiting the enigmatic question of why leaves are green. Plant Cell Physiol. 50: 684–697. doi.org/10.1093/pcp/pcp034

Tian, J., Zeng, X., Zhang, S., Wang, Y., Zhang, P., Lü, A., Peng, X. 2014. Regional variation in components and antioxidant and antifungal activities of *Perilla frutescens* essential oils in China. Ind. Crop Prod. 59: 69–79. doi.org/10.1016/j.indcrop.2014.04.048

Tripathi, S., Hoang, Q.T., Han, Y.J., Kim, J.I. 2019. Regulation of photomorphogenic development by plant phytochromes. Int. J. Mol. Sci. 20: 6165. doi.org/10.3390/ijms20246165

Verma, S.K., Gantait, S., Jeong, B.R., Hwang, S.J. 2018. Enhanced growth and cardenolides production in *Digitalis purpurea* under the influence of different LED exposures in the plant factory. Sci. Rep. 8: 18009. doi.org/10.1038/s41598-018-36113-9

Wang, L., Li, Y., Xin, G., Wei, M., Yang, Q., Mi, Q. 2017. Effects of red and blue light quality on nitrogen levels, activities and gene expression of key enzymes involved in nitrogen metabolism from leaves of tomato seedlings. Acta Hortic. Sin. 44: 768–776. doi: 10.16420/j.issn.0513-353x.2017-0002

Wang, T., Liu, Q., Wang, N., et al. 2021. Foliar arginine application improves tomato plant growth, yield, and fruit quality via nitrogen accumulation. Plant Growth Regul. 95: 421–428. doi.org/10.1007/s10725-021-00752-2

Wang, Y., Wang, D., Shi, P., Omasa, K. 2014. Estimating rice chlorophyll content and leaf nitrogen concentration with a digital still color camera under natural light. Plant Methods. 10: 36. doi.org/10.1186/1746-4811-10-36

Waterland, N.L., Moon, Y., Tou, J.C., Kim, M.J., Pena-Yewtukhiw, E.M., Park, S. 2017. Mineral content differs among microgreen, baby leaf, and adult stages in three cultivars of kale. Hort Sci. 52: 566–571. doi: 10.21273/HORTSCI11499-16

Winter, G., Todd, C.D., Trovato, M., Forlani, G., Funck, D. 2015. Physiological implications of arginine metabolism in plants. Front. Plant Sci. 6: 534. doi: 10.3389/fpls.2015.00534

Xiao, M., Li, Z., Zhu, L., Wang, J., Zhang, B., Zheng, F., Zhao, B., Zhang, H., Wang, Y., Zhang, Z. 2021. The multiple roles of ascorbate in the abiotic stress response of plants: antioxidant, cofactor, and regulator. Front. Plant Sci. 12: 598173. doi: 10.3389/fpls.2021.598173