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RESEARCH ARTICLE

Light supplementation and growing season affect the quality and antioxidant activity of lettuce

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ABSTRACT

The light spectrum plays a major role in regulating plant growth and development, influencing photosynthesis and photo-morphogenesis. The objective was to evaluate the weight and antioxidant activity of 'Lavinia' lettuce (*Lactuca sativa* L. var. *capitata* L.) plants under LED lights with different red:blue ratios (R:B) used as a supplement to ambient light in a greenhouse at different seasons. Five LED light supplemented treatments were used, white (W, as control, ratio blue:green:red:far-red (B:G:R:Fr) = 30:45:20:5; 0.7:1.0), blue (B = 50:20:20:10; R:B = 0.4:1.0), white-r (Wr = 25:30:40:5; 1.6:1.0), white-R (WR = 15:15:63:7; 4.2:1.0) and red (R = 10:10:75:5; 7.5:1.0) were applied in early autumn, late autumn and winter. Immediately after transplant into the hydroponic system, lettuces plants with 3-4 true leaves (5-6 cm root length) were treated with light treatments for 14 d and harvested. In early autumn, late autumn, and winter, the PAR range applied in ambient light and supplemental lights was between 361 to 495, 222 to 304, and 297 to 407 µmol m^{-2} s^{-1}, respectively. Lettuce showed the highest fresh leaf mass in early autumn compared to late autumn and winter due to the highest leaf number. In early autumn, only lettuce under the highest R:B (7.5:1.0) had a lower weight among the light treatments. Overall, lettuces supplemented with R:B between 1.6 (Wr) to 4.2 (WR) had significantly higher DM than the control (0.7:1.0). Total phenolic contents (TPC) and antioxidant capacity (AC) were highest in early autumn, followed by winter and late autumn. Light with the highest R:B (7.5:1.0) diminished TPC and AC in all growing season. This study showed that the quality of 'Lavinia' lettuce plants grown in early autumn under R:B between 1.6 and 4.2 improved DM, and higher red component decreased the antioxidant activity.

Key words: Antioxidant capacity, *Lactuca sativa*, LED lighting, total phenolic content, wavelength.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is an important leafy vegetable consumed mainly in fresh salads (Zhou et al., 2009); its demand has increased due to its crunchy texture, pleasant aroma, flavor, fresh appearance, and richness in phenolic compounds. Furthermore, lettuce is considered a healthy food because it is a good source of bioactive compounds such as phenolic compounds, vitamins A and B1, and minerals such as Fe and K (Carrasco and Sandoval, 2016). Biotic and abiotic factors can increase biologically active substances. For example, within abiotic factors, salinity can increase antioxidant compounds (Flores et al., 2022). On the other hand, it is widely known that light is one of the most important in modulating the accumulation of phytochemicals (Bian et al., 2014; Chen et al., 2014; Kwack et al., 2015) as well as the growth in vegetables (Bian et al., 2014; Hernández and Kubota, 2016). Lin et al. (2013) indicates that changes in the light spectrum evoke different morphogenetic and photosynthetic responses. These responses may vary among plant species and therefore become specific (Hernández and Kubota, 2016) and related to the stage of plant growth (Chen et al., 2014; Chang and Chang, 2014).

Under closed-type conditions, Hernández and Kubota (2016) demonstrated the shoot dry and fresh mass of cucumber seedlings decreased with the increase of blue photon flux when plants were irradiated with the combination of blue and red photon flux. In lettuce, Bian et al. (2016) observed that red-blue LED light (R: B = 4:1) was more effective than white LED light at the same photosynthetic photon flux in facilitating lettuce growth. In contrast, Lin et al. (2013) noted that the shoot and root fresh weights of lettuce, dry weight, and the shape of the plants treated with combined red and blue light were lower than those treated with combined red, blue, and white light and fluorescent lamps. However, when the fluorescent lamps were mixed with red or blue lights, lettuce seedlings and mature growth were promoted concerning fluorescent lamps alone (Chen et al., 2014). On the other hand, Li and Kubota (2009) indicated that baby leaf lettuce's fresh and dry weights increased significantly when white light was supplemented with far-red light compared to white light alone. Furthermore, red-blue light combined with other wavelengths can also favor the growth of lettuce plants. For example, Chang and Chang (2014) mentioned that the provision of red-blue-UV-A (5.3:3.7:1.0, respectively) and red-cyan-blue (4.3:3.0:2.7, respectively) LED irradiation during the vegetative phase could increase the fresh shoot mass of leaf lettuce. At the same time, red-green-blue (6.0:2.0:2.0, respectively) resulted in higher biomass attributes concerning the fluorescent lamp and light treatment with different combined red and blue (Lin et al., 2018).

On the other hand, light is one of the most critical environmental variables in regulating bioactive compounds (Bian et al., 2014). According to Lin et al. (2013), adapting the illumination spectra allows for controlling the nutritional quality of plants. In vegetables such as alfalfa and red radish sprouts, Kwack et al. (2015) showed that total phenolic content decreased with increasing red light intensity. Bantis et al. (2016) reported that two basil cultivars showed high total phenolic content under an LED light with blue, red, green, and UV wavelengths. Piovene et al. (2015) observed an increase in antioxidant compounds of the horticultural crop species under different red and blue LED light ratios. Similarly, Wojciechowska et al. (2015) observed the highest concentration of total phenols and radical detoxifying capacity in lamb's lettuce supplemented with red and blue light in a ratio of 9:1.

For lettuce plants, continuous light exposure at pre-harvest can effectively increase phytochemical concentrations (Bian et al., 2016). Pérez-López et al. (2018) observed that mild light stress imposed for a short time under ambient or elevated CO_2 concentration increased antioxidant capacity and phenolic content in two lettuce cultivars. According to Li and Kubota (2009), the phenolic content of baby leaf lettuce increased by 6% with supplemental R compared to those in the light control. Samuolienė et al. (2012a) found a higher content of total phenols in baby lettuce leaves supplemented with green LED light with peaks at 505 and 535 nm than blue LED light. On the other hand, the highest antioxidant capacity was found for the green light (peak at 535 nm) and the blue light (peak at 470 nm).

As many authors mentioned, spectrum light variation could enhance the health-promoting benefits of lettuce (Samuolienė et al., 2012a; 2012b; Bian et al., 2014). Nevertheless, studies on supplementing ambient light with LED lighting in various seasons' lettuce growth are limited. Therefore, this research aims to analyze how the supplementation of different light spectra at different seasons affects the weight, leaf number, and antioxidant activity in 'Lavinia' lettuce plants grown in hydroponics under a plastic greenhouse.

MATERIALS AND METHODS

Plant materials and growing conditions

The experiment was performed in a plastic chapel greenhouse (8 m × 33 m) and 5.8 m at zenith height equipped with a wet wall-and-fan cooling system at the Faculty of Agricultural Sciences at the Universidad de Chile (33°34' S, 70°38' W, Santiago, Chile). A polyethylene film on the top and sides 200 µm thick with more than 90% of global light transmission (Proamco, Santiago, Chile) was used to cover the greenhouse.

'Lavinia' lettuce (*Lactuca sativa* L. var. *capitata* L.) is an oak leaf with light green leaves, suitable for year-round production in hydroponics systems. It is a fast-growing cultivar (it takes approximately 30-40 d from transplant to harvest). 'Lavinia' lettuce seeds (Sakata, Bragança Paulista, São Paulo, Brazil) were sown in trays of 196 cells using peat moss (Kekkila professional, Protekta, Santiago, Chile) and perlite A6 (Harbolite Chile Ltda., Santiago, Chile) at a ratio of 1:1 (v:v). Seeded trays were kept in the greenhouse, and the seedlings were watered by capillarity, immersing the lower middle section of the tray in a sheet of water from a floating system until the root length reached 5 to 6 cm (25-30 d after sowing, DAS). The hydroponic system was carried out in plastic trays (40 cm × 30 cm × 6 cm) of 7.2 L capacity. Twelve plants were placed on an expanded polystyrene sheet for each plastic tray. The nutrient solution used was indicated by Sonneveld and Straver (1994). The pH and electrical conductivity were maintained between 5.8 to 6.0 and 2 mS cm^{-1}, respectively. The aeration was carried between 8 to 9 mg O_2 L^{-1} with air pumps (Sb-748, Sobo, Guangdong, China) of 3.5 L min^{-1}. The trays were placed in a 4-tier rack, open on both sides. The LED lights were installed randomly on each level at 20 cm above the plant's top. To avoid interference between treatments, plants were spaced 0.5 m apart, and a 3 mm thick wooden panel was placed between rack levels (Figure 1).

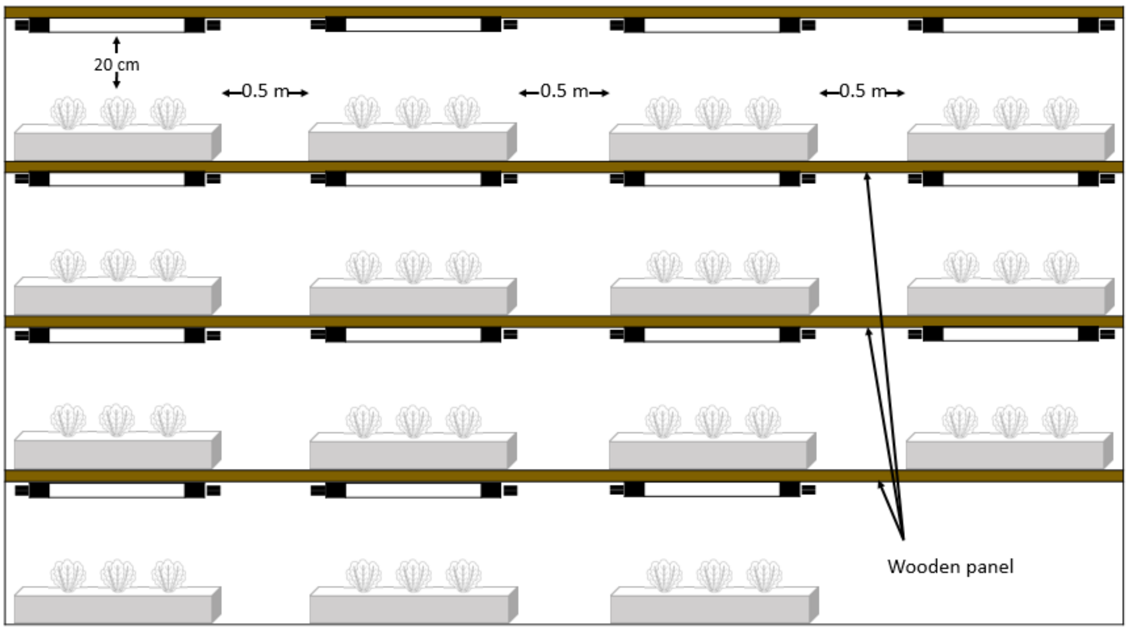

Figure 1. Diagram of the arrangement of the different LED light supplementation treatments under greenhouse conditions.

Light supplementation was carried out in three seasons during the year. The first season lasted from 2 to 16 April, coinciding with the beginning of autumn. The second season was from 24 May to 7 June, coinciding with late autumn. Finally, the third season corresponded between 10 and 24 August, coinciding with winter. During the experiment, photosynthetic active radiation (PAR) was the highest per day in early autumn, followed by winter and late autumn (Figure 2). During early autumn, late autumn, and winter, the mean ambient PAR was 290 ± 54.6, 130 ± 73.7, and 216 ± 72.6 µmol m^{-2} s^{-1}, respectively (Figure 2). In addition, the mean temperature during the experiment was most elevated in early autumn (20.0 ± 6.2 °C), followed by winter (16.2 ± 6.4 °C) and late autumn (12.5 ± 6.2 °C) (Table 1). Specifically, the average temperature in early autumn was 3.8 and 7.5 °C, higher than in winter and late autumn, respectively.

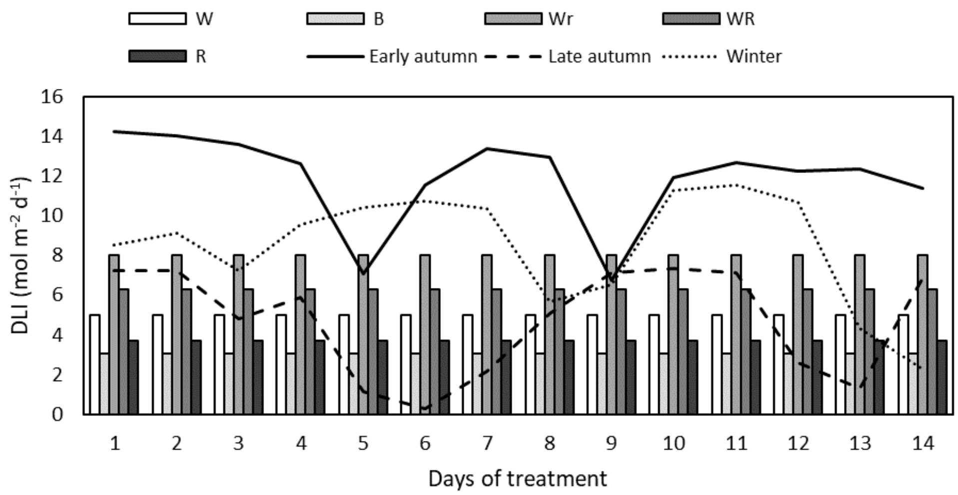

Figure 2. Daily light integral (DLI) for ambient light in the greenhouse for each season (lines) and each lamp for 14 d of the experiment. W: White as control; B: blue; Wr: white-r; WR: white-R; R: red.

Table 1. Mean temperature, minimum temperature, maximum temperature, and relative humidity inside the greenhouse during the experiment carried out in each season.

Season	Mean temperature	Minimum temperature	Maximum temperature	Relative humidity
	ºC	ºC	ºC	%
Early autumn (April)	20.0 ± 6.2	12.6 ± 1.5	29.6 ± 2.7	75.8 ± 2.6
Late autumn (May-June)	12.5 ± 6.2	6.1 ± 3.9	21.1 ± 5.3	74.6 ± 7.9
Winter (August)	16.2 ± 6.4	8.5 ± 3.1	24.5 ± 4.5	74.5 ± 6.0

Light spectrum treatments

All treatments considered different wavelengths of LED light to supplement ambient light inside the greenhouse. The characteristic of the light treatments were: White (W, as control) where ratio blue:green:red:far-red and ratio red:blue (B:G:R:Fr and R:B) were 30:45:20:5 and 0.7:1.0, respectively; blue (B), B:G:R:Fr = 50:20:20:10 and R:B = 0.4:1.0, respectively; white-r (Wr), B:G:R:Fr = 25:30:40:5 and R:B = 1.6:1.0, respectively; white-R (WR), B:G:R:Fr = 15:15:63:7 and R:B = 4.2:1.0, respectively; and red, (R) B:G:R:Fr = 10:10:75:5 and R:B = 7.5:1.0, respectively. White was considered as control because spectra were similar to the ambient light. Light supplementations were carried out for 14 d after the transplant (photoperiod 12:12 h, light from 08:00 to 20:00 h) until harvest. Plants were harvested as baby leaves with a maximum length of 10 cm (Battistoni et al., 2021). T5 LED tubes (18W; 110-240V) were used for treatments W, B, and R; while for treatments Wr and WR, Povi technology LED tubes, model PV02, and PV04 were used, respectively (18W; 100-240V). The mean PAR under treatments is shown in Table 2 for each season. The spectrum of each treatment (Figure 3) and the PAR were determined with the Lighting Passport Pro Essence spectroradiometer (AsenseTek, Taiwan, China). The PAR was measured at 20 cm under the LED light. The mean PAR under each treatment comprised LED lamps and ambient light radiation. Specifically, W, B, Wr, WR, and R LED lights had 116, 71, 185, 146, and 86 µmol m^{-2} s^{-1}, respectively. At the same time, ambient PAR was, on average, 290, 130, and 216 µmol m^{-2} s^{-1} in early autumn, late autumn, and winter, respectively, during the 14 d when light treatments were applied.

Table 2. Description of light supplementation treatments applied for the growth of 'Lavinia' lettuce plants for 14 d in each growing season. W: White as control; B: blue; Wr: white-r; WR: white-R; R: red. *Light spectrum of each treatment, values correspond to the percentage of blue:green:red:far-red component, respectively. **Means of photosynthetic active radiation during 14 d in each growing season.

			PAR** μmoles photons m^{-2} s^{-1}		
Light treatments	Red:Blue ratio	Spectrum light	Early autumn (April)	Late autumn (May-June)	Winter (August)
W (control)	0.7:1	30:45:20:5*	406	246	332
B	0.4:1	50:20:20:10	361	201	287
Wr	1.6:1	25:30:40:5	495	315	401
WR	4.2:1	15:15:63:7	436	276	362
R	7.5:1	10:10:75:5	376	216	302

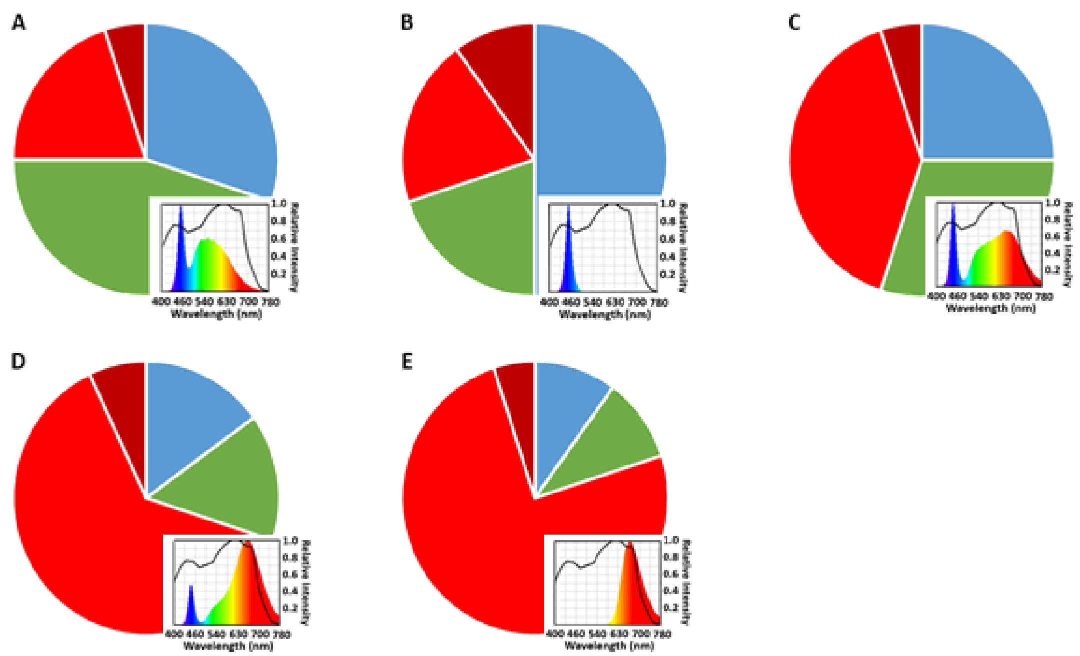

Figure 3. Characterization of the spectrum resulting from each light treatment by supplementing the ambient light of the greenhouse with each LED lamp. The proportion of the light's blue, green, red, and far-red components was similar between seasons. The small image represents the spectrum of the LED light alone used in each treatment. A: White light (W; R:B = 0.7:1.0; as control); B: blue light (B; R:B = 0.4:1.0); C: white-r (Wr; R:B = 1.6:1.0); D: white-R (WR; R:B = 4.2:1.0); E: red light (R; R:B = 7.5:1.0).

Plant measurements

Each evaluation was carried out on nine plants per treatment, meaning three plants were chosen for each replicate. All the measurements were made in plants grown each season independently after 14 d of transplant under different light treatments. The number of leaves was counted for each plant.

Fresh weight (FW) and DM percentage (DMP). The leaves of each plant were weighed using an analytical balance (RADWAG, AS/100/C/2, Radom, Poland), and fresh weight was recorded in grams.

For dry weight, first, leaves were weighed using an analytical balance (RADWAG, AS/100/C/2) and recorded as FW. Then, leaves were dried at 70 °C in an air circulating oven (LDOS50F, Daihan LabTech, Namyangju, Korea) until constant dried weight (DW). The DMP was determined using the following expression:

$$DMP = (1 - [(FW - DW)/FW]) \times 100$$

Antioxidant activity. Antioxidant activity was represented by total phenolic content and antioxidant capacity. Each of these evaluations was carried out on nine plants per treatment, i.e., three plants per replicate. In addition, antioxidant activity was measured as a plant's antioxidant status under different wavelengths.

Total phenolic content (TPC). The Folin-Ciocalteau reagent was used to quantify the total phenolic content (Swain and Hillis, 1959). About 100 µL homogenate (20 mg dry leaf powder with 70% methanol) was mixed with 200 µL 10% Folin solution. After 5 min equilibrium time, 800 µL Na_2CO_3 (0.7 M) solution was added to the extract, shaken, and incubated for 60 min at room temperature and darkness. The absorbance was measured at 765 nm (Asys UVM 340 microplate reader, Biochrom, Cambridge, UK). Total phenolic contents were calculated using a standard curve of gallic acid ranging from 0 to 2.4 mM and expressed as mg gallic acid equivalents (GAE) g DW^{-1}.

Antioxidant capacity (AC). The FRAP assay was used to measure antioxidant capacity (AC) (Benzie and Strain, 1996). About 20 mg leaf powder was mixed with 1 mL 70% methanol. Homogenates were centrifuged at 10500 g for 10 min at 4 °C (Hermle Brand centrifuge model Z326K, Wehingen, Germany) and filtered with 0.45 µm syringe filters. The FRAP solution included 100 mL acetate buffer (0.3 M, pH 3.6), 10 mL 10 mM 2,4,6-tripyridyl-*s*-triazine in 40 mM HCl and 10 mL 20 mM $FeCl_3 \cdot 6H_2O$ at 10:1:1 (v/v/v) and 600 µL reagent were warmed up to 37 °C and mixed with 20 µL homogenate sample into the microplate wells for 30 s. As a control, the absorbance of FRAP solution was measured after 30 min of the reaction at 593 nm (Asys UVM 340 microplate reader). A calibration curve was obtained using a series of Trolox ranging from 0 to 1600 mM and expressed as Trolox equivalent antioxidant capacity (mg TEAC g DW^{-1}).

Experimental design

The experiment was set up in a completely randomized design with a factorial structure of 5 × 3 with three replicates. The statistical model of ANOVA was:

$$y_{ijk} = \mu + L_i + S_j + L{\times}S_{ij} + e_{ijk}$$

where y_{ijk} represents the k^{th} repetition (k = 1 to 3) at the i^{th} level of the Light spectrum factor (i = 1 to 5) and j^{th} level of the Season factor (j = 1 to 2), L_i and S_j represent main effects of the Light spectrum and Season, respectively, $L{\times}S_{ij}$ represents the interaction effect between main factors, and e_{ijk} defines the experimental error term for experimental unit y_{ijk}.

Each replicate was an independent plastic tray, and each tray had 12 lettuce plants. The first factor was the LED light spectrum, which had five levels: White, blue, white-r, white-R, and red. The second factor corresponded to the season: Early autumn, late autumn, and winter. Results were reported as the mean ± standard deviation (SD) values. Data were evaluated by ANOVA and differences among the means compared by Tukey's test ($P < 0.05$). Statistical analyses were performed on the SAS OnDemand for Academics program (SAS Institute, Cary, North Carolina, USA).

RESULTS

Fresh weight

The fresh weight of 'Lavinia' lettuce was affected by Light×Season interaction (Table 3). The supplemented red with the highest red:blue ratio (7.5:1) in early autumn caused the lowest fresh weight in lettuce plants (8.9 ± 0.9 g) compared to the other light treatments. Specifically, fresh weight was 25% lower than lettuce plants produced under control (W), whose red:blue ratio was 0.4:1. There were nonsignificant differences between the light treatments and the control (W) in late autumn and winter (Table 4). On the other hand, lettuce showed the highest fresh leaf mass in early autumn (11.6 ± 2.4 g) and was significantly different from lettuce plants produced in late autumn (3.4 ± 0.6 g) and winter (6.8 ± 0.9 g) (data not shown). Overall, lettuce fresh weights in early autumn were 71.1% and 42.5% higher than in late autumn and winter, respectively, while late autumn was 50.4% lower than winter (Table 4).

Table 3. Summary of the ANOVA for the five variables analyzed in the study. Df: Degrees of freedom; MS: mean square; DMP: DM percentage.

Source of variation	df*	Fresh weight		DMP		Leaf number		Antioxidant capacity		Phenolic total content	
		MS	p-Value	MS	p-Value	MS	p-Value	MS	p-Value	MS	p-Value
Light (L)	4	4.17	0.0075	23.28	< 0.0001	0.11	0.7914	1915.17	< 0.0001	192.34	< 0.0001
Season (S)	2	275.86	< 0.0001	1.64	< 0.0031	14.27	< 0.0001	8609.09	< 0.0001	457.32	< 0.0001
L × S	8	3.87	< 0.0027	2.35	< 0.0001	0.16	0.7729	207.33	< 0.0002	54.12	< 0.0001
Residual error	44	0.98	-	0.23	-	0.26	-	36.52	-	4.18	-

Table 4. Fresh weight of 'Lavinia' lettuce grown under different light treatments. W: White, control; B: blue; Wr: white-r; WR: white-R; R: red. *Light spectrum of each treatment, values correspond to the percentage of blue:green:red:far-red components, respectively. **Different lowercase letters in columns indicate significant differences among light treatment levels inside each season level, according to Tukey's multiple range test ($p \leq 0.05$). Different uppercase letters in rows indicate significant differences among season levels inside each light treatment level according to Tukey's multiple range test ($p \leq 0.05$). Mean (n = 3) ± SD.

Supplemental light	Red:Blue ratio	Spectrum light	Fresh weight (g) Early autumn (April)	Late autumn (May-June)	Winter (August)
W (control)	0.7:1.0	30:45:20:5*	11.8 ± 1.2aA**	3.3 ± 0.7aC	6.2 ± 1.5aB
B	0.4:1.0	50:20:20:10	12.5 ± 2.0aA	3.9 ± 0.4aC	6.5 ± 1.3aB
Wr	1.6:1.0	25:30:40:5	13.5 ± 2.1aA	3.8 ± 0.7aC	6.6 ± 1.3aB
WR	4.2:1.0	15:15:63:7	13.0 ± 2.2aA	2.9 ± 0.9aC	8.0 ± 1.0aB
R	7.5:1.0	10:10:75:5	8.9 ± 0.9bA	3.3 ± 0.4aB	6.9 ± 1.3aA

Dry matter percentage (DMP)

A significant interaction between light and season was observed for lettuce DMP (Table 3). In all seasons, lettuce plants supplemented with WR (R:B = 4.2:1.0) had a significantly higher DMP than W (R:B = 0.7:1.0). Besides, in late autumn and winter, lettuce plants supplemented with Wr (R:B = 1.6:1.0) promoted significantly higher DMP than the control (R:B = 0.7:1.0). Among the seasons, lettuce plants supplemented with B produced higher DMP in early autumn with 5.4 ± 2.0% compared to late autumn with 3.6 ± 0.4%. Meanwhile, the control had higher DMP in early autumn (6.3 ± 1.2%) and winter (5.9 ± 1.5%) compared to late autumn (4.6 ± 0.7%). Moreover, lettuce plants treated with Wr (R:B = 1.6:1.0) had higher DMP in late autumn (7.9 ± 0.7%) and winter (8.3 ± 1.3%) compared to early autumn (5.8 ± 2.1%) (Table 5). On the other light treatments, nonsignificant differences were observed among seasons. It is interesting to mention that under lights with R:B between 1.6:1.0 and 4.2:1.0, the lettuce plants accumulated a higher DMP in late autumn and winter, where climatic conditions were lower than optimal for this species and compared to early autumn (Table 5).

Table 5. Dry matter percentage of 'Lavinia' lettuce grown under different light treatments. W: White, control; B: blue; Wr: white-r; WR: white-R; R: red. *Light spectrum of each treatment, values correspond to the percentage of blue:green:red:far-red components, respectively. **Different lowercase letters in columns indicate significant differences among light treatment levels inside each season level, according to Tukey's multiple range test ($p \leq 0.05$). Different uppercase letters in rows indicate significant differences among season levels inside each light treatment level according to Tukey's multiple range test ($p \leq 0.05$). Mean (n = 3) ± SD.

			DMP (%)		
Supplemental light	Red:Blue ratio	Spectrum light	Early autumn (April)	Late autumn (May-June)	Winter (August)
W (control)	0.7:1.0	30:45:20:5*	6.3 ± 1.2bA**	4.6 ± 0.7bB	5.9 ± 1.5bA
B	0.4:1.0	50:20:20:10	5.4 ± 2.0bcA	3.6 ± 0.4bB	4.6 ± 1.3cAB
Wr	1.6:1.0	25:30:40:5	5.8 ± 2.1bcB	7.9 ± 0.7aA	8.3 ± 1.3aA
WR	4.2:1.0	15:15:63:7	7.8 ± 2.2aA	8.7 ± 0.9aA	8.4 ± 1.0aA
R	7.5:1.0	10:10:75:5	4.7 ± 0.9cA	4.7 ± 0.4bA	5.4 ± 1.3bcA

Leaf number

The analysis of the results showed that the number of leaves was affected only by the season factor (Table 3). In early autumn, 7.1 ± 0.8 leaves plant^{-1} were significantly higher than in late autumn (5.7 ± 0.6 leaves plant^{-1}) and winter (5.4 ± 0.5 leaves plant^{-1}). These data represented that the number of leaves in early autumn was significantly higher than in late autumn and winter, by 31.5% and 24.6%, respectively. Nonsignificant differences were observed between late autumn and winter (Figure 4).

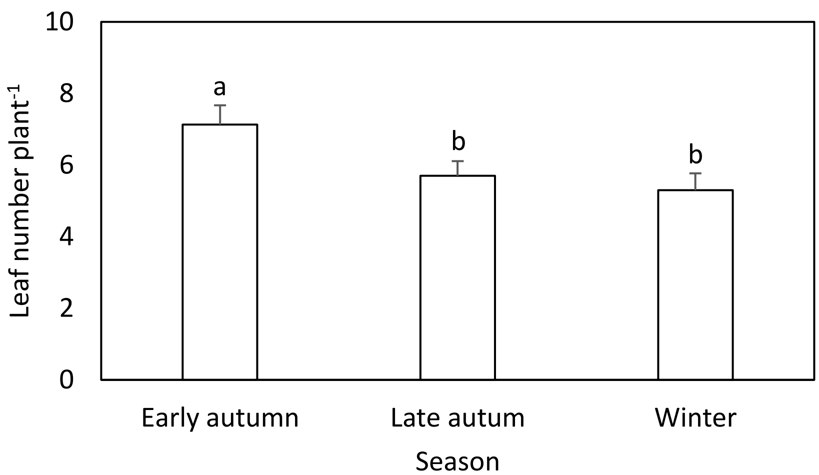

Figure 4. Leaf number per plant of 'Lavinia' lettuce grown in different seasons. Different lowercase letters indicate significant differences among season levels according to Tukey's multiple range test ($p \leq 0.05$).

Antioxidant activity

The interaction of factors affected the total phenolic content (Table 3). During early autumn and winter, lettuce plants are grown under light with a higher R:B (R) of 7.5:1.0 achieved 19.9% and 12.7% less than the W (control) during the same seasons. Furthermore, in early autumn, the TPC under WR (R:B = 4.2:1.0) was significantly higher than in late autumn (Table 6). At the same time, the TPC under B (R:B = 0.4:1.0) enhanced considerably in order from early autumn, winter, and late autumn (Table 6).

It is noteworthy that despite low temperatures and radiation in the late autumn and winter seasons, lettuce plants increased total phenolic content when light's red component increased from R:B = 0.4:1.0 to R:B = 1.6:1.0 (Table 6).

Table 6. Total phenolic content of 'Lavinia' lettuce grown under different light treatments. W: White, control; B: blue; Wr: white-r; WR: white-R; R: red. *Light spectrum of each treatment, values correspond to the percentage of blue:green:red:far-red components, respectively. **Different lowercase letters in columns indicate significant differences among light treatment levels inside each season level, according to Tukey's multiple range test ($p \leq 0.05$). Different uppercase letters in rows indicate significant differences among season levels inside each light treatment level according to Tukey's multiple range test ($p \leq 0.05$). Mean (n = 3) ± SD.

Supplemental light	Red:Blue ratio	Spectrum light	Phenolic total content (mg GAE g^{-1} DW)		
			Early autumn (April)	Late autumn (May-June)	Winter (August)
W (control)	0.7:1.0	30:45:20:5*	31.2 ± 1.2bA**	26.6 ± 1.5abB	28.3 ± 0.7aAB
B	0.4:1.0	50:20:20:10	40.8 ± 1.1aA	21.7 ± 1.3cC	27.7 ± 0.4aB
Wr	1.6:1.0	25:30:40:5	41.1 ± 2.1aA	28.3 ± 1.3aB	28.8 ± 0.7aB
WR	4.2:1.0	15:15:63:7	27.2 ± 2.2bcA	22.0 ± 1.0bcB	25.6 ± 0.9aAB
R	7.5:1.0	10:10:75:5	25.0 ± 0.9cA	13.9 ± 1.3dB	24.7 ± 0.4aA

The effect of supplemental light on antioxidant capacity was season-dependent (Table 3). Notably, in early autumn and winter, lettuce plants under control (W) (R:B = 0.7:1.0) reached the highest antioxidant capacity compared to the other light treatments, except under Wr (R:B = 1.6:1.0). In contrast, the lowest antioxidant capacity was observed in lettuce plants under R (R:B = 7.5:1.0) in all seasons compared to the other light treatments (Table 7). On the other hand, the treatments W (control), B, WR, and Wr in early autumn triggered significantly higher antioxidant capacity in lettuce plants than in winter and late autumn. In contrast, R provoked a lower antioxidant capacity in late autumn than in early autumn and winter (Table 7). Similar to the total phenolic content (Table 6), antioxidant capacity increased when the red light component increased until R:B reached 1.6:1.0 (Table 7) despite lower temperature and radiation in late autumn and winter than in early autumn.

Table 7. Antioxidant capacity of 'Lavinia' lettuce grown under different light treatments. W: White, control; B: blue; Wr: white-r; WR: white-R; and R: red. *Light spectrum of each treatment, values correspond to the percentage of blue:green:red:far-red components, respectively. **Different lowercase letters in columns indicate significant differences among light treatment levels inside each season level, according to Tukey's multiple range test ($p \leq 0.05$). Different uppercas letters in rows indicate significant differences among season levels inside each light treatment level according to Tukey's multiple range test ($p \leq 0.05$). Mean (n = 3) ± SD. TEAC: Trolox equivalent antioxidant capacity.

Supplemental light	Red:Blue ratio	Spectrum light	Antioxidant capacity (mg TEAC g DW^{-1})		
			Early autumn (April)	Late autumn (May-June)	Winter (August)
W (control)	0.7:1.0	30:45:20:5*	117.4 ± 1.5aA**	50.3 ± 1.2aC	85.4 ± 0.7aB
B	0.4:1.0	50:20:20:10	93.5 ± 1.3bcA	59.9 ± 2.0aB	70.2 ± 0.4bB
Wr	1.6:1.0	25:30:40:5	106.6 ± 1.3abA	53.9 ± 2.1aC	84.7 ± 0.7aB
WR	4.2:1.0	15:15:63:7	90.3 ± 1.0cA	46.2 ± 2.2aC	65.1 ± 0.9bB
R	7.5:1.0	10:10:75:5	62.9 ± 1.3dA	31.5 ± 4.1bB	59.6 ± 0.4bA

DISCUSSION

Fresh weight and DM percentage

According to Kubota (2020), plant growth is affected by environmental factors such as temperature, light spectrum, and intensity. The current study indicated that the interaction between light and season produced the most significant effect on fresh weight (Table 3). The higher fresh weight of 'Lavinia' lettuce plants in the early autumn compared to late autumn and winter is due to the higher number of leaves observed during that season (Figure 4) and the environmental conditions (Table 1; Figures 2 and 3). Early autumn would be a more suitable season for growing lettuce plants, as Maynard and Hochmuth (2006) mentioned that the optimum growth temperature of lettuce is 18 °C. In addition, solar radiation in early autumn was higher than in late autumn and winter adding more PAR under the different light levels used in this experiment (Figures 2 and 3). The highest PAR could favor the photosynthesis process that subsequently affects all other physiological processes related to photosynthates transport in plants. Zhang et al. (2018) and Pennisi et al. (2020) indicated that a PAR of 250 µmol m^{-2} s^{-1} would be adequate for the growth and high quality of 'Gentilina' and 'Ziwei' lettuce plants. Therefore, a low PAR shown in late autumn could be considered as insufficient quality for 'Lavinia' lettuce plants retarding the growth (Lin et al., 2013). On the other hand, Hytönen et al. (2017) observed that biomass accumulation depends on photosynthesis driven by light absorption of chlorophyll pigments. According to Marín-Ortiz et al. (2020), the absorption spectrum of photosynthetic pigments has two absorption ranges, between 430 and 453 nm (blue region) and 642 and 662 nm (red region). Therefore, the photosynthetic process is favored at those wavelengths and under a more balanced spectrum, such as white LED light, as it could better penetrate the plant canopy than red-blue LED light (Lin et al., 2013). The above would explain the lower fresh weight of 'Lavinia' lettuce plants grown under R with a higher R:B ratio and, therefore, an unbalanced spectrum compared to the other light treatments with a lower R:B ratio and more balanced spectrum during early autumn. On the other hand, the highest fresh weight under light treatments in early autumn does not depend on the number of leaves but is probably due to a change in leaf area. According to Lin et al. (2013), the lettuce leaf area increased significantly with the red-blue-white compared to the red-white. Li and Kubota (2009) mentioned that the leaf length and width of baby leaf lettuce increased significantly under white light supplemented with far-red light (R:B = 1.2:1.0) compared to white light plus red light, whose R:B ratio was 4.6:1.0. Nevertheless, some studies have shown that red light has a marked positive effect on plant growth and development when applied alone or combined with fluorescent lamps or ambient lighting (Battistoni et al., 2021; Hernández-Adasme et al., 2022). Battistoni et al. (2021) observed that spinach leaf length increased significantly under ambient light supplemented with red light in the greenhouse. In contrast, Flores et al. (2021) noted a higher leaf area in lettuce and endive under light with a spectrum with a higher red component.

Wavelengths in various leafy vegetables can affect DM (Flores et al., 2021; Lara et al., 2021; Hernández-Adasme et al., 2022). Our results indicated that the effect of light on DMP depended on seasons (Table 3). Overall, DMP enhanced when the red component increased until R:B was 4.2:1.0 (Table 5). The environmental conditions were unexpected since, during the winter, they were better than in the late autumn, which may explain the results obtained under the light treatments in each season (Table 1; Figures 2 and 3). According to Colonna et al. (2016), DMP may be influenced by other factors such as plant species and light intensity. In particular, leaf DM content was significantly higher when leafy vegetables were harvested at low (200-400 µmol m^{-2} s^{-1}) compared to high PAR (800-1200 µmol m^{-2} s^{-1}). In our research, lettuce plants were subjected to low intensity, according to Colonna et al. (2016), which varied between 222 and 495 µmol m^{-2} s^{-1} among all light treatments. Nevertheless, the spectrum was relevant because the highest DMP was observed only under Wr and WR. Kim et al. (2004) indicated that lettuce 'Waldmann's Green' showed higher dry weight when grown under LED light whose R:B ratio was 4.1:1.0 compared to light with R:B ratios of 5.3:1.0; 0.4:1.0; and 1.6:1.0. Lin et al. (2018) found that biomass parameter of lettuce 'Korea' was higher under the treatments with an R:B ratio = 2.3:1.0 compared with a light in which R:B was 0.4:1.0 under the same intensity (150 µmol m^{-2} s^{-1}; 16 h of light). Hernández and Kubota (2016) observed a decrease in fresh and dry weights of cucumber seedlings under monochromatic red light. Moreover, noted raised with an increasing red component within the combined blue-red light until the R:B ratio was 1.0:1.0 (100 µmol m^{-2} s^{-1}; 18 h of light). Similar results were noted in the same species that grew in a greenhouse with supplemental LED lighting under low daily light integral conditions (60 ± 13.9 µmol m^{-2} s^{-1}) (Hernández and Kubota, 2014). Hernández-Adasme et al. (2022) observed an increase in the dry weight of green leaf lettuce under light with R:B = 0.4:1.0 and monochromatic blue light, resulting in an increase in DMP compared to ambient light. In contrast, spinach plants grown under ambient light enriched with red light improved fresh and dry weight (Battistoni et al., 2021). On the other hand, Flores et al. (2021) demonstrated that DMP in lettuce and endive increased when the plants grew under high red component light with an intensity of 100 µmol m^{-2} s^{-1}. Therefore, according to literature results, a high proportion of red component improved the biomass of plants, although the effect is species dependent. According to Xu et al. (2016), lights of 400-520 nm (blue) and lights of 610 to 720 nm (red) contribute most to the photosynthesis process. In particular, Wang et al. (2016) found that the photosynthetic leaf process increased with decreasing R:B ratio until 1.0:1.0, associated with increased stomatal conductance, a significant increase in stomatal density, and a slight decrease in stomatal size. On the other hand, the photosynthetic rate is directly related to carbohydrate production, affecting DMP (Min et al., 2021). Hence, lights with an R:B ratio between 1.6:1.0 (Wr) and 4.2:1.0 (WR) could be suitable for DM accumulation in 'Lavinia' lettuce.

Antioxidant activity

According to Nicolle et al. (2004), the total phenols can account for more than 60% of the antioxidant capacity. In addition, the antioxidant capacity was linearly correlated with the phenolic content (Samuolienė et al., 2012b; Damerum et al., 2015). Therefore, the phenolic compounds may explain the high antioxidant capacity (AC) observed in early autumn and late autumn under light with R:B = 1.6:1.0 (Wr) (Tables 6 and 7). On the other hand, the lowest AC is noted in all seasons under the light with the highest R:B (R) (Table 7). However, it must be considered that the phenolic profile can be influenced by intrinsic and external factors such as agronomic practical (Santos et al., 2014; Flores et al., 2022) and environmental conditions (Santos et al., 2014). For example, in greenhouse conditions, Samuolienė et al. (2012b) noticed that the accumulation of total phenolic compounds changed depending on the growing seasons in two lettuce varieties. Specifically, it was 62.6% higher in early and late winter than in autumn for 'Multibaby' and 61.6% to 70.2% for 'Thumper' in early and late winter, respectively. Regarding antioxidant capacity, Samuolienė et al. (2012a) observed a contradictory effect of supplemental blue and green LED lighting on the antioxidant properties of red, green, and green light varieties of baby leaf lettuce during the cultivation time of the year. Precisely, the antioxidant capacity of green lettuce and light green lettuce was higher in autumn and full winter than in late winter. In contrast, red lettuce was higher in early winter than in late winter, which, in turn, was higher than autumn. Our research showed that light's effect on antioxidant capacity depended on the growing seasons (Table 3). Antioxidant capacity was higher in early autumn, followed by winter, and finally in late autumn under all light treatments, which was also observed for total phenolic content (Tables 6 and 7). The environmental conditions observed during the winter were unforeseen, as the PAR and temperature were higher than in late autumn (Figure 2; Table 1) due to a higher number of cloudless days (Meteochile, 2022). The PAR radiation can significantly affect molecules related to antioxidant activity in plants. For example, Dębski et al. (2017) noted that increased PAR from 180 to 360 µmol m^{-2} s^{-1} contributed to increased flavonols in the cotyledons of common buckwheat seedlings; meanwhile, Idris et al. (2018) mentioned that increase in PAR leads to the accumulation of flavonoids. Flavonoids are secondary metabolites that exhibit antioxidant properties and may be responsible for antioxidant activity. However, it must be considered that light's effect on phenolic compounds depends on the specific type of phenolic compound and plant species (Thoma et al., 2020). Several phenolic compounds are accumulated in lettuce, such as quercetin, quercetin-3-*O*-glucuronide, kaempferol, quercitrin, and rutin under high intensity (Pérez-López et al., 2018). Furthermore, Samuolienė et al. (2012a) observed an increase in vitamin C accumulation during a lighter growing period (late winter) in green and light green leaf lettuce. Other compounds may also influence antioxidant activity. According to Damerum et al. (2015), phenolic, carotenoids, and chlorophyll were essential contributors to the antioxidant potential in lettuce. Tocopherols are also molecules found in different lettuce varieties under light treatments during the autumn (November) (Samuolienė et al., 2012a). The accumulation of these compounds may explain the higher total phenolic content and antioxidant capacity found in early autumn, followed by winter, compared to late autumn.

Also, spectrum light plays an essential role in phytochemical compounds. Total phenolic content and antioxidant capacity were high under light with an R:B ratio = 1.6:1.0 (Wr). In contrast, they diminished progressively as the red component increased and the blue part decreased in light treatments (Tables 6 and 7). Samuolienė et al. (2012a) observed that DPPH free-radical scavenging capacity significantly increased under supplemental 470 and 535 nm LED lighting in red, green, and light green lettuce plants cultivated in a greenhouse. In the growth chamber, Mireles et al. (2020) indicated that the percentage of antioxidant activity determined through the DPPH in lettuce plants (Romaine type) was lower under red light than blue, green, and white lights (150 lux; 12:12 h photoperiod) applied for 6 d before the harvest. Son and Oh (2013) found that antioxidant capacity gradually increased when enhanced the proportion of blue component in combined red and blue light (171 µmol m^{-2} s^{-1}; 12 h of light) from monochromatic red light (100% red) to R:B = 1.1:1.0 (47% blue-53% red) in ‘Sunmang’ (red leaf) and Grand Rapid TBR (green leaf) lettuces. Likewise, Son and Oh (2015) observed that with the enhanced red component of light (173 µmol m^{-2} s^{-1}; 12:12 h photoperiod), the AC of ‘Sunmang’ lettuce plants decreased. This study indicated that antioxidant capacity and total phenolic content decreased when the red component increased and blue decreased, becoming lowest in light, whose R:B was 7.5:1.0 (R) in all seasons. According to Thoma et al. (2020), the optimal blue light for accumulating carotenoids and flavonols, representative of antioxidant capacity, depends on the specific phenolic compound and plant species. Furthermore, Cuong et al. (2018) noted that blue light upregulated phenylpropanoid and flavonoid biosynthetic genes (*PAL*, *C4H*, *4CL*, *CHS*, *CHI*, *F3H*, *FLS*, *DFR*, and *3GT*) compared to white or red light in bitter melon seedlings. It could explain the higher antioxidant capacity of ‘Lavinia’ lettuce under Wr independently of the seasons. On the other hand, the lowest antioxidant activity found under R (R:B = 7.5:1.0) could occur due to a decrease in phenylpropanoid and flavonoid formation pathways by red light (Cuong et al., 2018).

CONCLUSIONS

The results underline that the effect of light on the weight, DM percentage, and antioxidant parameters of ‘Lavinia’ lettuce plants depends on the growing season. The DM percentage tended to increase when the red component of light was raised. The red:blue ratio between 1.6:1.0 and 4.2:1.0 promoted a higher percentage of DM in all seasons. On the contrary, the antioxidant activity decreased when the red component increased, becoming the lowest under the highest red:blue ratio (7.5:1.0). On the other hand, the fresh weight of ‘Lavinia’ lettuce plants increased in early autumn due to better environmental conditions such as temperature and radiation. On the other hand, most of the variables respond to the interaction of light with the growing season; hence, the effect of light or season alone cannot be considered.

Author contribution

Conceptualization: C.H-A., H.S., V.M., V.E. Methodology: C.H-A., J.S-R. Formal analysis: C.H-A., J.S-R. Investigation: C.H-A. Resources: V.E. Data curation: C.H-A., J.S-R. Writing-original draft: C.H-A. Writing-review & editing: C.H-A., H.S., J.S-R., V.M., V.E. Supervision: V.E. Project administration: V.E. Funding acquisition: V.E. All co-authors reviewed the final version and approved the manuscript before submission.

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