https://doi.org/10.71252/2177-34912026730014
ISSN: 2177-3491

Impact of varying light spectra on the biochemical and physiological parameters of lettuce (*Lactuca sativa* L.)[1]

Luciano Tartaro[2], *Jorge Luiz Barcelos Oliveira*[3], *Fernanda Espíndola Assumpção Bastos*[2*], *Elinton Soares Pontes*[2], *Simone Daniela Sartorio de Medeiros*[4], *Rosete Pescador*[2]

[1] This work is derived from the first author's master's thesis.

[2] Universidade Federal de Santa Catarina, Departamento de Fitotecnia, Florianópolis, SC, Brazil. luciano.tartaro@hotmail.com; elintonpon@gmail.com; rosete.pescador@ufsc.br

[3] Universidade Federal de Santa Catarina, Centro de Ciências Agrárias, Departamento de Engenharia Rural, Florianópolis, SC, Brazil. j.barcelos@ufsc.br

[4] Universidade Federal de Santa Catarina, Centro Tecnológico, Departamento de Informática e Estatística, Florianópolis, SC, Brazil. simone.dsm@ufsc.br

*Corresponding author: feabastos@hotmail.com

Editors:
Ricardo Marenco
Marihus Altoé Baldotto

Submitted: November 27th, 2025.
Accepted: July 28th, 2026.

ABSTRACT

The search for plants that can increasingly adapt to diverse environmental conditions has intensified in recent years. In order to understand the biochemical and physiological responses of lettuce (*Lactuca sativa* L.) to light, plants of this species were grown under monochromatic LEDs emitting blue, warm white, blue + red, and red light, as well as under a comparative condition of natural light. The experiment was conducted in a randomized block design, with five lighting treatments, four cultivation cycles as blocks, and six plants per experimental unit. The production of total soluble sugars, chlorophyll a, chlorophyll b, total chlorophylls, carotenoids, photosystem II quantum efficiency, and the SPAD index was analyzed. Among the biochemical analyses conducted, sugar production was reduced only in plants exposed to warm white LEDs. Regarding chlorophyll a, total chlorophylls, and carotenoids, monochromatic blue and blue + red LEDs stood out, whereas chlorophyll b production did not differ among treatments under the environmental conditions of this experiment. Based on physiological analyses, photosystem II quantum efficiency was higher in plants irradiated with blue and natural light, while the SPAD index was highest under blue + red, blue, and natural light. Blue and blue + red spectra promoted superior photosynthetic performance and pigment accumulation, indicating that these wavelengths are more suitable for optimizing physiological quality of lettuce grown under controlled environments.

Keywords: chlorophylls, SPAD, photosystem II efficiency, indoor.

INTRODUCTION

Plants are living organisms capable of adapting to the environmental conditions to which they are exposed, generating adaptations at the cellular level aimed at surviving in their environment. The quantity of light (intensity and photoperiod) and quality (spectral composition) affect plant growth and physiology and interact with other environmental parameters and cultivation factors in determining plant behavior. More than providing energy for photosynthesis, light also dictates specific signals that regulate plant development, form, and metabolism, in photomorphogenesis, driven by light colors.[1] The complex phenomenon of various metabolic responses can be modulated according to light intensity, quality, periodicity, and direction.[2] When the problem is the absence or low intensity of specific wavelengths, the symptoms are typically etiolation, reduced leaf blade elongation, among others. Periodicity, in turn, acts mainly on the plant's circadian cycle, identifying "day and night," and plants exposed to insufficient or absent dark periods may exhibit disturbances in their circadian rhythm and, under severe conditions, necrosis of apical meristems.[3] The direction of light allows the plant to identify shading, dawn, and dusk symptoms, as in these cases, the amount of far-red wavelength is higher, which generates responses at the phytochrome level.[2] Over the years, tools have been developed to quantify and subsequently mitigate the effects of stress on plants. Chlorophyll fluorescence analysis is a technique that not only quantifies photosynthetic tolerance mechanisms to various stimuli but also predicts chloroplast activity and responses under different growth conditions, whether optimal or unfavorable.[4] The optimal quantum yield of photosystem II (Fv/Fm ratio) for plants ranges from 0.75 to 0.85.[5] Values below this range may indicate stress or photoinhibition and may trigger metabolic and photoprotective responses. When the plant is in a comfortable situation, 97% of the light is used for photosynthesis; 2.5% is transformed into heat; and 0.5% is re-emitted as fluorescence.[5] Fluorescence is the re-emission of absorbed light at a longer wavelength (approximately 668 nm), corresponding to a lower energy state. Deficiency symptoms can be identified by the pale and chlorotic coloration of the leaves, culminating in a reduced photosynthetic rate.[7] In higher plants, the most important photosynthetic pigments are chlorophylls a and b and carotenoids.[6] The chlorophyll pigment is one of the molecules responsible for light absorption and conversion into chemical energy, in the form of ATP and NADPH.[8] Carotenoids, in turn, are accessory pigments that absorb blue light and channel it to the photosynthesis process, optimizing the wavelength bands not captured by photosystem I and II.[6] Since their main absorption is in the 400 to 500 nm range, plants exposed to this wavelength range have a greater capacity to synthesize this compound, as found by authors such as Lin et al.[9] The quantification of chlorophylls and carotenoids is a parameter that can help identify plants with higher production potential. The Soil and Plant Analysis Development (SPAD) is an efficient device for instantaneously and accurately measuring chlorophyll and other pigments, in order to maintain proper plant health, measuring the amount of light transmitted at 650 and 940 nm wavelengths,[10] being considered an indirect method of quantifying leaf chlorophyll, used as a comparison to destructive chemical analyses. In addition to chlorophyll content, another important factor to consider in the final quality of leafy vegetables is the total soluble sugars (TSS), which are molecules formed at the end of the Calvin Benson cycle and play a fundamental role as an energy reserve in plants.[6] The physicochemical properties of plants can reflect the process of material exchange and metabolism in the plant, and metabolism is closely related to the growth and development of organisms. A higher TSS content contributes to a more pleasant taste of lettuce. In a study conducted by Dai et al.[11] it was found that a light intensity of 210 μmol m^{-2} s^{-1} significantly affected the content of total soluble sugar, vitamin C, nitrate, and free amino acids. Previous studies have investigated sugar production in response to different light wavelengths, but the results have been inconsistent. Chen et al[12] and Zhang et al[13] observed higher values in plants exposed to red light. On the other hand, Alves[14] reported that plants of the Brassica genus had higher content when exposed to blue light, and Mandacaru plants (*Cereus jamacaru* L.) achieved better results with the combination of red and blue light. Therefore, although most studies mention red light as the most relevant, there is a lack of consensus on the optimal wavelength to maximize sugar production in lettuce cultivation.

Lettuce (*Lactuca sativa* L.) is one of the most widely cultivated leafy vegetables worldwide and is frequently used as a model species in controlled-environment agriculture due to its short cycle, high commercial value, and rapid physiological responses to environmental

changes. Previous studies have shown that light spectrum directly affects lettuce morphology, biomass accumulation, photosynthetic efficiency, and nutraceutical quality.[3]

Studies conducted under artificial lighting demonstrated that blue wavelengths stimulate chlorophyll synthesis and stomatal regulation, whereas red light may enhance biomass accumulation but can induce etiolation when applied alone. Despite these findings, inconsistencies remain regarding the optimal spectral composition for maximizing biochemical quality in lettuce.[3,11] Thus, this study aimed to quantify biochemical variations in lettuce plants exposed to different light spectra based on the analysis of photosynthetic pigments and soluble sugars.

MATERIAL AND METHODS

Seeds of the BSAC0055 cultivar (Blue Seeds, Holambra, São Paulo, Brazil) were used in all treatments. Thirteen days after emergence (DAE), seedlings were transferred to the experimental environment, where they were exposed to different lighting conditions. Seedlings were standardized to three fully expanded leaves before being subjected to the respective experimental conditions.

The experiment was conducted with five treatments, four with exposure to artificial light (Figure 1) and one to natural lighting conditions in a greenhouse. The artificial light treatments were: blue light (T1), warm white light (T2), blue + red combination in a 1:1 ratio (T3), and red light (T4). For this, LED panels (LumiGrow®, Emeryville, California, USA) were used, equipped with warm-white, blue, and red LEDs, and integrated with a individual potentiometers for each LED channel, allowing modulation of light intensity and wavelength.

The LED experiments were conducted in an indoor environment with temperature control, maintained at 25 °C (±2) via air conditioning, and with no external light interference. For the natural light treatment, a greenhouse covered with a 200 µm thick diffusing polyethylene film was used. The average temperature inside the greenhouse was 23 °C, with a photoperiod of approximately 13 hours and an average light intensity of 950 µmol m² at 12:00 hours.

The nutrient solution used in the experiment is presented in Table 1, based on the solution proposed by Furlani[15], with added nitrogen, aiming for better plant development.

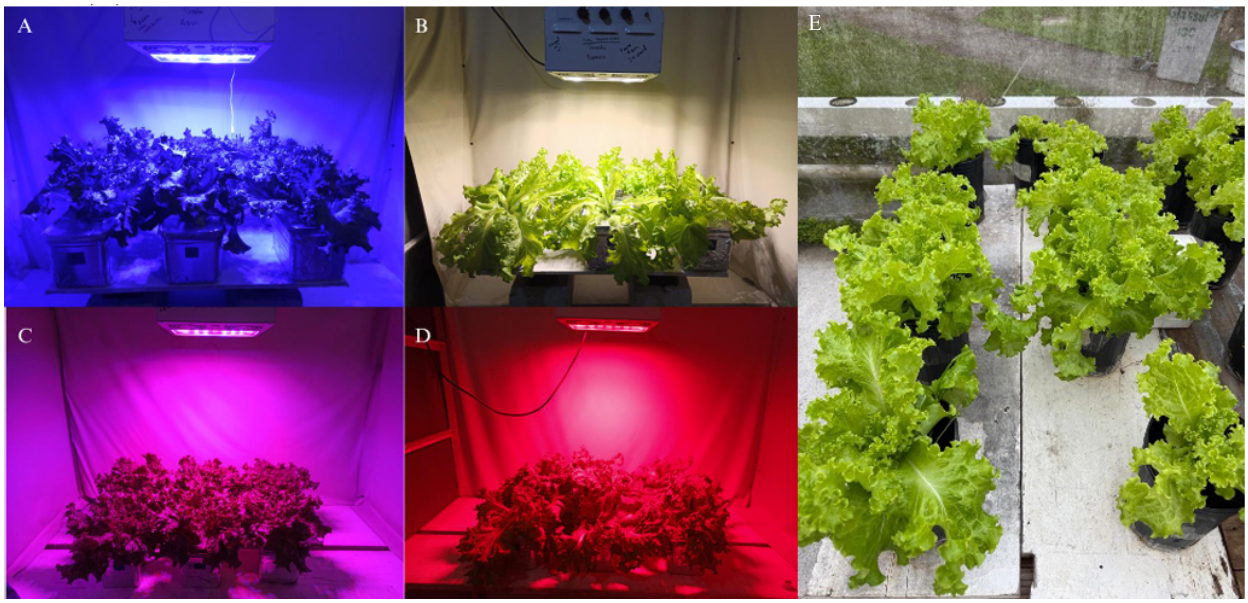

Figure 1. Artificial light treatments to which lettuce plants (*Lactuca sativa* L.) were subjected. A) Blue light (T1), B) White light (T2), C) Blue + red light (T3), D) Red light (T4) and E) Natural light.

Table 1. Concentrations of nutrients in the nutrient solution used in the experiment. Data in mg L^{-1}

Macronutrients							Micronutrients						
NO_3	NH_4	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Mo	Zn	Ni
188,9	16,6	8,0	252,6	139,5	42,8	48,1	0,617	0,614	2,700	0,614	0,138	0,240	0,122

Source: Furlani[15]

In addition to the aforementioned nutrients, Silamol® was used as a source of potassium silicate (K_2SiO_3), at a dosage of 3 mL per 1000 liters of solution, to provide greater resistance to pathogens that may affect the crop, as well as better root development.

The light intensity was established based on a daily light integral (DLI) of 13 mol m^{-2} d^{-1} and a photoperiod of 18 h d^{-1}.[3] Based on these parameters, the photosynthetically active photon flux was adjusted to 200 µmol m^{-2} s^{-1}.

The color quantification of each treatment was performed with a spectroradiometer, determining the wavelength peaks at 665 nm for red and 442 nm for blue. In the combined treatment (T3), both peaks were considered, and in the white light treatment (T2), there was a mixture of multiple wavelengths.

Physiological parameters were analyzed with readings throughout the cultivation cycles, observing increases or decreases in values relative to the baseline readings, i.e., the day of cycle implementation, when all were under the same environmental conditions.

The variables analyzed were the SPAD index, average levels of total soluble sugars (TSS), chlorophyll a (CHL_A), chlorophyll b (CHL_B), total chlorophylls (CHL_T), carotenoids (CAR) and fresh shoot mass (FSM). The following equipment was used for the measurements: a portable Mini-PAM fluorometer (Heinz Walz GmbH), a SPAD-502 chlorophyll meter (Konica Minolta Sensing), a Dist4 portable conductivity meter (Hanna Instruments), and a graduated cylinder.

The Mini-PAM fluorometer is equipment capable of measuring, among other parameters, chlorophyll a fluorescence. To verify the plant's status regarding fluorescence, readings were taken on days 0, 7, and 13. To enable fluorescence quantification to enable fluorescence measurements, the leaves were dark-adapted for at least 1 h before the readings, with light cessation at least one hour before readings. At the time of reading, darkness was maintained, with only the light from the computer screen on in the location.

Each plant was removed from its respective environment and carefully positioned for the readings to take place. Leaves from the middle third were marked and clamped in three locations, and the data were observed in real time to check for congruence. If a vein or damaged leaf was clamped, values that diverged from the others were discarded and new readings were taken. Finally, the pot was returned to its respective environment.

The maximum fluorescence (Fm), initial fluorescence (F0) and the quantum yield of photosystem II (Fv/Fm ratio) were obtained automatically using the Win-Control 3 software, belonging to the equipment used. The light pulse used to saturate the photosystems was 7500 µmol m^{-2} s^{-1}.

The SPAD index, responsible for showing the plant's status regarding environmental and nutritional conditions, was analyzed on days 0, 2, 5, 7, 9, and 13. The readings were performed at the plant cultivation site, in an illuminated environment. Leaves from the middle third were selected, identified, and read. As this is a parameter that often has high variation, triplicate readings were performed and the mean recorded; if a value diverged from the others within the same plant, it was discarded. Data were recorded and the increase or decrease in values according to the cycle progression was observed.

Biochemical analyses were performed only after the cultivation cycles were completed. Their main objective was the quantification of some photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophylls and carotenoids) and the total soluble carbohydrates impregnated in the plant tissues.

Photosynthetic pigments were determined at the end of the cultivation period, after 40 days of cultivation. The methodology used was that of Hiscox and Israelstam[16] taking into account some modifications for lettuce culture. Samples of 50mg of new and fully expanded leaves from each plant were collected, placed in test tubes wrapped in aluminum foil, and 5.0 mL of dimethyl sulfoxide (DMSO) was poured into them. Then, the tubes were closed and kept in a water bath at 70 °C for one hour, until complete dissolution of chlorophyll. Aliquots of 200 microliters were taken and placed in an Elisa plate and read in a microplate reader (brand Molecular Devices, model SpectraMax Paradigm) at wavelengths of 663 nm for chlorophyll a (CHL-A), 645 nm for chlorophyll b (CHL-B) and 470 nm for carotenoids (CAR). Only DMSO was used as a blank.

For the quantification of chlorophylls, in mg of chlorophyll per gram of fresh weight (mg g^{-1} FW), the equations proposed by Hiscox and Israelstam[16] were used.

$$\text{Chlorophyll } a = * \frac{((11.75 * A663) - (2.35 * A645)) * 50}{500}$$

$$\text{Chlorophyll } b = \frac{((18.61 * A663) - (3.96 * A645)) * 50}{500}$$

$$\text{Total Chlorophyll} = \text{Clorofila } a + \text{Clorofila } b$$

$$\text{Carotenoids} = \frac{\frac{((1000 * A470) - (2.27 * Chl\_a) - (81.4 * Chl\_b)}{227} * 50}{50}$$

For the determination of total soluble sugars (TSS), the phenol-sulfuric acid extraction methodology proposed by Dubois et al.[17] was followed. Leaves from the middle third

of the plants were used, removed at the end of the crop cycle and placed in 50 mL Falcon tubes, stored in an ultra-freezer at -80 degrees. The samples were stored until the last block was harvested and then destined for lyophilization in a freeze dryer from JJ Científica, model LJ UP, remaining inside it until the vacuum pressure became constant, indicating total moisture removal. After lyophilization, the samples were previously stored in a desiccator containing silica, to prevent any moisture retention.

For sugar determination, the samples were ground in a grinder, which allowed the samples to have low granulometry, similar to maceration with liquid nitrogen. The sample mass was determined according to the dry weight equivalent per gram of fresh weight. For T1, 52.20 grams were used; for T2, 39.70 grams; for T3, 44.20 grams; for T4; 48.30 grams and T5, 53.60 grams.

The samples were inserted into test tubes along with 2mL of 80 % ethanol (v/v), prepared before analysis and kept cold. The tubes were placed in a water bath at 70 °C, for a period of 5 minutes. Subsequently, they were centrifuged at 3000 rpm for 10 min. The supernatant was collected and filtered using a graduated pipette, syringe and glass fiber filter, with the process repeated in triplicate. The obtained filtrate (approximately 6mL) was made up to adjusted to a final volume of 10 mL with 80 % ethanol. All samples were placed in screw-cap tubes and stored in a freezer at -20 °C until the next step.

A glucose standard solution was prepared, using 10mg/mL of D-Glucose (Sigma-Aldrich brand). The standard solution was diluted in scaled doses according to table 2, and the absorbance was read in a UV-VIS spectrophotometer.

For the quantification of total soluble carbohydrates, aliquots of 50 µl of the previously prepared extract were used. The aliquots were inserted into test tubes and 450 µl of distilled water, 500 µl of 5% phenol and 2500 µl of concentrated sulfuric acid (96%) were added, in this respective order. The tubes were left to rest for 20 minutes, then vortexed and read in a spectrophotometer at a wavelength of 490 nm. The carbohydrate content was estimated according to the glucose equivalent, prepared in the standard curve stage.

The experiment was conducted in a randomized complete block design, with four cultivation cycles considered as blocks. Each experimental plot consisted of six plants, resulting in 20 experimental plots and a total of 120 plants. For destructive analyses, all six plants from each experimental plot were evaluated, and the mean value was considered for statistical analysis. The obtained data were subjected to analysis of variance and when the results were significant, the Tukey multiple comparison test ($p < 0.05$) was applied.

RESULTS AND DISCUSSION

Analysis of variance revealed significant effects of light spectrum on total soluble sugars (TSS), chlorophyll a (CHL_A), total chlorophyll (CHL_T), carotenoids (CAR), maximum fluorescence (Fm), Fv/Fm ratio, and SPAD index ($p \leq 0.05$). In contrast, chlorophyll b (CHL_B) was not significantly affected by the treatments. Coefficients of variation ranged from 1.73 to 12.46%, indicating satisfactory experimental precision.

According to the results obtained (Table 3), it is observed that plants exposed to blue light and blue + red light stood out for the majority of the variables analyzed. Thus, the importance of the highlighted wavelengths is evident, forming plants with a greener hue, higher chlorophyll content, compared to the others, and thus favored mass accumulation.

Although numerically higher TSS values were observed under blue and blue + red spectra, these treatments did not differ statistically from the intermediate groups. Significant differences were observed only between treatments represented by distinct Tukey groupings. The obtained results did not fully align with expectations, since sugar synthesis in lettuce is influenced by the type and intensity of light.[6]

Table 2. D-Glucose dilutions for absorbance definition. Data expressed in microliters (µl)

Dose (mg/mL)	D-Glucose	Distilled water	Phenol 5%	Sulfuric acid	Final volume
0	0	500	500	2500	3500
10	10	490	500	2500	3500
20	20	480	500	2500	3500
40	40	460	500	2500	3500
60	60	440	500	2500	3500
80	80	420	500	2500	3500

Table 3. Mean levels of total soluble sugars (TSS), chlorophyll a (CHL_A), Chlorophyll b (CHL_B), total chlorophylls (CHL_T), carotenoids (CAR) and fresh shoot mass (FSM), and coefficient of variation (CV) of each variable

	T1	T2	T3	T4	T5	CV (%)
TSS	174,08 ab	171,42 b	174,62 ab	180,49 a	175,51 ab	1,73
CHL_A	0,671 a	0,525 bc	0,636 ab	0,509 c	0,541 bc	9,07
CHL_B	0,111 a	0,122 a	0,124 a	0,125 a	0,120 a	12,46
CHL_T	0,782 a	0,648 c	0,760 ab	0,635 c	0,661 bc	7,11
CAR	2,39 a	1,90 c	2,30 ab	1,83 c	2,05 bc	6,61
FSM	68,86 c	51,67 d	109,32 a	107,37 a	78,97 b	3,82

T1 (blue), T2 (white), T3 (blue + red 1:1), T4 (red) and T5 (natural light); * Means followed by the same letter in the row do not differ from each other by the Tukey test at 5% probability

In the literature, some authors have found that sugar accumulation in plants does not follow a single pattern, with one wavelength being more specific for the synthesis of this compound. Plant sugar levels are influenced by phytochromes and red light.[13,18,19] However, in addition to red, other wavelengths have also shown prominence: Blue and White;[9] blue and red in *Cereus jamacaru* and Blue in *Brassica campestris* L. and *Brassica oleracea* var. *acephala.*[14] The latter author further reiterates that blue may be prominent due to the action of metabolic pathways for detoxifying reactive oxygen species (ROS).

For T2 plants, sugar synthesis was lower than the other treatments; however, the low morphological performance observed, coupled with the lower values of photosystem II efficiency (Fv/Fm ratio), resulted in a plant without ideal conditions for the accumulation of secondary compounds, and diverges from that of Lin et al,[9] for example.

Chlorophylls (CHL_A, CHL_B and CHL_T) and carotenoids (CAR). Regarding chlorophyll a (CHL_A), it is observed that plants subjected to monochromatic blue light (T1) and blue + red (T3) accumulated the most of this molecule (0.671mg. g FW^{-1} and 0.636mg. g FW^{-1}, respectively), not differing from each other, but showing statistical difference from the others. Plants from T2, T3 and T5 showed no significant statistical difference and produced average levels.

However, the lowest synthesis was observed in T4 plants, with 0.509mg. g FW^{-1}. Regarding chlorophyll b (CHL_B), plants exposed to all treatments were statistically equivalent, but in decreasing order of accumulation they are listed: T4, with 0.125mg. g FW^{-1}; T3, with 0.124mg. g FW^{-1}; T2, with 0.122mg. g FW^{-1}; T5, with 0.120mg. g FW^{-1}; and finally T1 with 0.111mg. g FW^{-1}. Regarding total chlorophylls, plants exposed to treatments T1 and T3 synthesized the most, with 0.782mg. g FW^{-1} and 0.760mg. g FW^{-1}, respectively, not differing from each other, but differing statistically from the others. However, T3 and T5 do not differ from each other, with T5 synthesizing 0.661mg. g FW^{-1}.

Similarly, T5 does not differ from T2 and T4, which obtained 0.648mg. g FW^{-1} and 0.635mg. g FW^{-1}. Given the results found for these pigments in the present study, it can be verified that the light environment to which the plants are exposed interferes with the production and accumulation of pigments in leaf tissues. In fact, several authors have reported on this statement. In experiments conducted by Zhang et al.[13] and Amoozgar et al.[20] working with lettuce (*Lactuca sativa* L.) and Cabral,[21] working with two species of Brazilian orchids: *Gomesa flexuosa* and *Epidendrum denticulatum*, it was possible to verify that the plants altered the chlorophyll composition according to the lighting conditions to which they were exposed.

Regarding chlorophyll a and total chlorophylls (Sum of CHL_A and CHL_B), the aforementioned authors obtained higher results in plants exposed to a combination of red and blue colors or monochromatic red.

However, in the present experiment, blue plants (T1) led in the presence of chlorophylls and even though in the same statistical class, plants exposed to red and blue (T3) obtained about 5.21% less CHL_A and 2.81% less CHL_T. The plant's life stage can interfere with the amount of chlorophylls, for example, blue light being superior at 17 days, but at 45 days the highest composition shifted to plants irradiated by red LEDs.[22] Blue light was also superior to red light in inducing chlorophyll synthesis in primary barley leaves (*Hordeum vulgare* L.)[23] Both situations corroborate the results of the present study. The synthesis of chlorophylls is mediated by a complex chain of metabolic transformations, which convert glutamic acid into chlorophylls, with some steps mediated by light.[24]

Protochlorophyllide is an intermediate in chlorophyll biosynthesis, and its conversion to chlorophyll is influenced by light-dependent processes involving blue and red wavelengths,[25] and which can interfere with the amount of chlorophyll in a plant. Similarly, the stress suffered by plants exposed to light is reflected in the amount of chlorophyll,[26] which tells us that the amount of chlorophyll present is not always dependent on the plant's ability to synthesize it, but rather on the plant's ability not to degrade it under stress situations.

With the present experiment, it can be verified that plants exposed to red light, white light and environmental condition (T5) obtained lower values for CHL_A and CHL_T. For the first two mentioned, it is observed that they were treatments affected by lower photosynthetic yield, corroborated by the Fv/Fm ratio, and which may have culminated in the degradation of chlorophylls and reduction of the ratio in these treatments. In turn, in T5 the shorter photoperiod and consequent lower DLI may have caused the lower indices among the analyzed variables.

In plants, there are two chemical forms, chlorophyll a and b, usually present in a 3:1 ratio. The difference between the two forms of chlorophyll lies in the presence of a methyl group on carbon C3 for chlorophyll a, while in chlorophyll b a formyl group was found in the same position.

The fact that there are no different accumulations of chlorophyll b among the treatments is not unprecedented, as already seen in *Gomesa flexuosa* and *Epidendrum denticulatum* Bard. Rodr.[21]

Plants exposed to T1 and T3 accumulated the highest carotenoid contents, reaching 2.39 and 2.30 mg g^{-1} FW, respectively, differentiating them from the other treatments. Plants exposed to the blue + red light condition (T3), however, did not differ from T5 plants, which accumulated 2.05mg g FW^{-1}. In T5, in turn, the plants were equal to those of T2, with 1.90mg g FW^{-1} and those of T4, with 1.83mg g FW^{-1}.

Carotenoids are pigments involved in light capture and photoprotection, particularly in the absorption of blue wavelengths.[6] Therefore, it is expected that plants exposed to these wavelengths may increase the synthesis of these compounds and experience less damage associated with light stress. Higher carotenoid levels in plants exposed to blue light have been reported.[14,22,27] Johkan et al.[22], for example, observed the highest values in plants exposed to blue light, particularly up to 17 days of cultivation. In contrast, higher carotenoid levels have also been reported in plants exposed to combined blue and red light.[20,28] Overall, these studies indicate that the increased production of carotenoids may be associated with the induction of photoprotective responses by specific wavelengths, particularly blue light.

The blue spectral range, because it contains higher energy value, favors energy absorption by accessory pigments and chlorophyll b.[29] Carotenoids also act in the dissipation of excess light, preventing oxidative stress, through the release of heat and/or chlorophyll a fluorescence,[30] which can be corroborated by the data analyzed by MINIPAM, where the blue light treatment obtained the highest ratios - but within the standard reiterated by Bolhàr-Nordenkampf and Öquist[5] - in photosynthetic efficiency (Fv/Fm ratio).

Table 4 presents the results obtained after the physiological analyses performed on lettuce plants exposed to the lighting conditions. The data refer to the average of the readings taken on the last day of cultivation (13th day), and were performed for the quantum efficiency of photosystem II (Fv/Fm ratio) and for the SPAD index (Soil and Plant Analysis Development).

Based on the data presented in Table 4, it is evident that plants exposed to treatments T1 and T5 exhibited the highest values for the ratio between variable fluorescence and maximum fluorescence (Fv/Fm), at 0.830 and 0.833, respectively. However, plants subjected to treatment T1 did not differ statistically from those under T3, occupying an intermediate position with a value of 0.820 and showing no divergence from the remaining treatments. The lowest values in the experiment were recorded for plants in

Table 4. Means of quantum efficiency of photosystem II (MINIPAM) and SPAD index (SPAD); and the coefficient of variation (CV) for each of the variables

Variable	Treatment					CV (%)
	T1	T2	T3	T4	T5	
MINIPAM	0,830 ab*	0,805 c	0,820 b	0,804 c	0,833 a	0,69
SPAD	24,89 a	14,65 c	25,01 a	17,47 b	23,85 a	3,5

#T1 (blue), T2 (white), T3 (blue + red 1:1), T4 (red) and T5 (natural light); *Averages followed by the same letter in the line do not differ from each other by Tukey's test at 5% probability

treatments T2 and T4, which differed from the others and showed mean Fv/Fm values of 0.805 and 0.804, respectively, under both light conditions.

The quantum efficiency of photosystem II is inferred from the relationship between variable fluorescence (Fv) and maximum fluorescence (Fm), and is a widely used parameter for assessing plant photosynthetic health, including in lettuce. Optimal Fv/Fm ratios typically range from 0.75 to 0.85, with values below this threshold potentially indicating inhibition of photosystem II activity.[5] Therefore, based on the Fv/Fm values obtained, it can be observed that despite distinct phenotypic responses and the presence of etiolation in plants from treatments T2 and T4, no substantial reduction in photosystem II efficiency occurred, as the values remained around 0.8. This is in agreement with the findings of Marani et al[32] in his study on lettuce grown under LED lighting. At the end of the 13-day cultivation cycle, the lowest values of photosynthetic quantum efficiency were observed under T2 and T4, corresponding to white and red light, respectively. The absence of blue light inhibits cryptochrome activity and reduces chlorophyll biosynthesis, resulting in decreased photochemical efficiency in plants not exposed to this wavelength.[31] The quantum yield of energy dissipation (φDo) and the energy dissipation flux per reaction center (DioRC) showed higher values under red LEDs, in agreement with the reduction in Fv/Fm observed under this lighting condition.[32] For plants subjected to T2 (warm white light), the lower values may be attributed to the light intensity and color temperature to which the plants were exposed. Marani et al[32] reported higher Fv/Fm values under blue, blue + red, and white LEDs; however, their experiment employed a light intensity of 400 ± 20 μmol m^{-2} s^{-1} – twice that of the present study – and a different plant species (Alternanthera brasiliana Kuntze), which may account for the discrepancy.

Lower Fv/Fm values negatively affected morphological and biochemical traits (as shown in Table 3), reducing most measured variables except for shoot height—where etiolation led to an increase in size—and chlorophyll b, which did not differ statistically among treatments. Treatments T1 and T5 were those in which plants experienced the lowest stress levels within the photosynthetic apparatus, as evidenced by the highest Fv/Fm ratios. Moreover, plants under T1 showed the highest carotenoid synthesis, while those under T5 exhibited intermediate values. This observation is relevant, as carotenoids play a critical role in photoprotection against excessive light stress, as highlighted by Lazzarini[30] and broadly supported in the literature.[6] Regarding the lighting condition combining blue and red wavelengths (T3), Centofante[33] reported that this combination was the most effective when evaluating *Campomanesia pubescens* (Mart. ex DC.) O. Berg. However, it is essential to emphasize that experimental conditions and species-specific characteristics may have influenced those results, which were not reproduced in the present study. With respect to the SPAD index, the light treatments that yielded the highest values were T3 (25.01), T1 (24.89), and T5 (23.85), which did not differ statistically. Treatment T2 exhibited the lowest SPAD index (14.65). On the final day of the cultivation cycle (13th day), lettuce plants under T1, T3, and T5 displayed increased SPAD values compared to the initial measurement. The SPAD index indirectly estimates chlorophyll content and correlates with the nitrogen (N) concentration within plant tissues.[34] Notably, plants subjected to these same treatments experienced the least photosynthetic stress, a factor that may influence SPAD readings in cases of damage to the photosynthetic apparatus. In T1, the correlation between the SPAD index and chlorophyll content—particularly chlorophyll a—revealed that the highest values were obtained under blue light. Furthermore, these plants exhibited greater nutrient solution uptake, likely resulting in enhanced nutrient accumulation in leaves and, consequently, increased chlorophyll synthesis. Shin et al.[35] reported similar findings, achieving a SPAD index of 25 ± 1 in lettuce grown under blue LEDs similar to those used in this study, although superior results were obtained under combined red and blue LEDs. In the present experiment, plants exposed to blue + red light did not exhibit significant advantages over the other lighting conditions. It is worth noting that the aforementioned authors used a 50% higher light intensity than that employed here, which may explain the observed differences. Conversely, plants under T5 were exposed to natural light, as previously described for other variables, without control over light intensity or spectral quality. Nevertheless, the lighting conditions to which these plants were subjected supported healthy growth and enabled valid comparison with the other two treatments, which did not differ statistically from T5. When analyzing T2 and T4, their values were consistently lower than those of the remaining treatments. Kobayashi et al.[36] investigated lettuce growth under red, blue, and fluorescent light treatments and reported that plants exposed to red light exhibited the lowest

SPAD indices. In the present study, treatments T2 and T4 were likewise negatively affected by their respective light conditions, resulting in reduced photosynthetic rates. These effects may be associated with chlorophyll degradation, which adversely impacts the SPAD index. Chlorophylls are primarily degraded by several enzymes – including chlorophyllase, Mg-dechelatase, pheophytinase, peroxidase, and chlorophyll oxidase – that catalyze chlorophyll breakdown, leading to color loss, textural changes, and decreased nutritional value in plant tissues and food products.[37] Indeed, the SPAD and chlorophyll (CHL_A and CHL_T) data corroborate one another, as white and red light treatments yielded the lowest values for both parameters.

CONCLUSIONS

For lettuce plants of the BSAC0055 cultivar, the spectral quality of light decisively influences physiological and biochemical parameters. Blue light and the blue + red combination promoted higher quantum efficiency of photosystem II, higher SPAD index values, and increased levels of chlorophyll a, total chlorophyll, and carotenoids. White light resulted in the lowest numerical TSS content; however, this treatment did not differ statistically from the intermediate groups. Thus, using artificial lighting with adjusted spectra, especially involving blue and blue + red light, is a promising strategy to optimize photosynthetic performance and physiological quality of plants in controlled cultivation.

ACKNOWLEDGMENTS, FINANCIAL SUPPORT AND FULL DISCLOSURE

The authors thank the Laboratory of Plant Physiology and the Department of Crop Science at the Federal University of Santa Catarina for providing the facilities and technical support necessary to conduct this research.

This research received no specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

The authors declare that they have no financial or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

Conceptualization: Luciano Tartaro, Rosete Pescador.

Data curation: Luciano Tartaro, Fernanda Espíndola Assumpção Bastos, Elinton Soares Pontes.

Formal analysis: Luciano Tartaro.

Funding acquisition: Rosete Pescador.

Investigation: Luciano Tartaro, Elinton Soares Pontes.

Methodology: Luciano Tartaro, Jorge Luiz Barcelos Oliveira, Elinton Soares Pontes.

Project administration: Luciano Tartaro, Jorge Luiz Barcelos Oliveira, Rosete Pescador.

Software: Luciano Tartaro.

Resources: Rosete Pescador, Jorge Luiz Barcelos Oliveira.

Supervision: Rosete Pescador, Jorge Luiz Barcelos Oliveira.

Validation: Luciano Tartaro, Jorge Luiz Barcelos Oliveira, Rosete Pescador, Simone Daniela Sartorio de Medeiros.

Visualization: Rosete Pescador.

Writing – original draft: Luciano Tartaro.

Writing – review & editing: Jorge Luiz Barcelos Oliveira, Fernanda Espíndola Assumpção Bastos, Simone Daniela Sartorio de Medeiros, Rosete Pescador.

REFERENCES

1. Paradiso R, Proietti S. Light-quality manipulation to control plant growth and photomorphogenesis in greenhouse horticulture: the state of the art and the opportunities of modern LED systems. J Plant Growth Regul. 2022;41(2):742-80. https://doi.org/10.1007/s00344-021-10337-y
2. Kerbauy GB. Fisiologia vegetal. Rio de Janeiro: Guanabara Koogan; 2004.
3. Silva LM. Management of lighting in indoor cultivation of lettuce (Lactuca sativa L.) [dissertation]. Campinas: Agronomic Institute of Campinas; 2021.
4. Jan MF, Li M, Liaqat W, Altaf MT, Liu C, Ahmad H, et al. Fluorescência da clorofila: uma ferramenta inteligente para o melhoramento do milho. Cereal Res Commun. 2025;53(2):617-48. https://doi.org/10.1007/s42976-024-00573-9
5. Bolhàr-Nordenkampf HR, Öquist G. Chlorophyll fluorescence as a tool in photosynthesis research. In: Hall DO, Scurlock JMO, Bolhàr-Nordenkampf HR, Leegood RC, Long SP, editors. Photosynthesis and production in a changing environment: a field and laboratory manual. Dordrecht: Springer Netherlands; 1993. p. 193-206. https://doi.org/10.1007/978-94-011-1566-7_12
6. Taiz L, Zeiger E, Møller IM, Murphy A. Plant physiology and development. 6th ed. Porto Alegre: Artmed; 2017.
7. Larcher W. Ecofisiologia vegetal. 3rd ed. São Carlos: RiMa; 2006.
8. Mendili M, Khadhri A. Chlorophylls: the verdant world of photosynthetic pigments. In: Sharma S, Sharma B, Singh D, editors. Microbial colorants: chemistry, biosynthesis and applications. Hoboken: Wiley; 2025. p. 223-39. https://doi.org/10.1002/9781394287888.ch9

9. Lin KH, Huang MY, Huang WD, Hsu MH, Yang ZW, Yang CM. The effects of red, blue, and white LEDs on the growth, development, and edible quality of hydroponically grown lettuce (Lactuca sativa L. var. capitata). Sci Hortic. 2013;150:86-91. https://doi.org/10.1016/j.scienta.2012.10.002

10. Dadhich A, Kumar R, Dhiman M, Sharma L, Sharma MM. Comparative assessment of chlorophyll content in various plants using SPAD-502 plus. Recent Adv Mater Sci Comput Tech. 2023;2723:020025. https://doi.org/10.1063/5.0139328

11. Dai M, Tan X, Ye Z, Ren J, Chen X, Kong D. Optimal light intensity for lettuce growth, quality, and photosynthesis in plant factories. Plants. 2024;13(18):2616. https://doi.org/10.3390/plants13182616

12. Chen XL, Xue XZ, Guo WZ, Wang LC, Qiao XJ. Growth and nutritional properties of lettuce affected by mixed irradiation of white and supplemental LED light. Sci Hortic. 2016;200:111-8. https://doi.org/10.1016/j.scienta.2016.01.007

13. Zhang X, He D, Niu G, Yan Z, Song J. Effects of environment lighting on the growth, photosynthesis, and quality of hydroponic lettuce in a plant factory. Int J Agric Biol Eng. 2018;11(2):33-40. https://doi.org/10.25165/j.ijabe.20181102.3

14. Alves CFG. Germination, biochemistry and initial growth of Cereus jamacaru seedlings under different light qualities [dissertation]. Arapiraca: Federal University of Alagoas; 2019.

15. Furlani PR. Instruções para o cultivo de hortaliças de folhas pela técnica de hidroponia NFT. Campinas: Instituto Agronômico; 1998. (IAC Documents, 168).

16. Hiscox JD, Israelstam GF. A method for the extraction of chlorophyll from leaf tissue without maceration. Can J Bot. 1979;57(12):1332-4. https://doi.org/10.1139/b79-163

17. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related compounds. Anal Chem. 1956;28(3):350-6.

18. Rehman M, Ullah S, Bao Y, Wang B, Peng D, Liu L. Light-emitting diodes: whether an efficient source of light for indoor plants? Environ Sci Pollut Res. 2017;24(32):24743-52. https://doi.org/10.1007/s11356-017-0333-3

19. Chen XL, Wang LC, Li T, Yang QC, Guo WZ. Sugar accumulation and growth of lettuce exposed to different lighting modes of red and blue LED light. Sci Rep. 2019;9:6915. https://doi.org/10.1038/s41598-019-43498-8

20. Amoozgar A, Mohammadi A, Sabzalian MR. Impact of light-emitting diode irradiation on photosynthesis, phytochemical composition and mineral element content of lettuce cv. Grizzly. Photosynthetica. 2017;55(1):85-95. https://doi.org/10.1007/s11099-016-0216-8

21. Cabral NN. How do spectral qualities of LED lights applied in the in vitro cultivation of Brazilian orchids influence morphophysiological and biochemical parameters during acclimatization? [dissertation]. Florianópolis: Federal University of Santa Catarina; 2021.

22. Johkan M, Shoji K, Goto F, Hashida S, Yoshihara T. Blue LED irradiation improves seedling quality and growth after transplanting in red leaf lettuce. HortScience. 2010;45(12):1809-14. https://doi.org/10.21273/HORTSCI.45.12.1809

23. Bukhov NG, Drozdova IS, Bondar VV, Mokronosov AT. Blue, red and blue+red light control of chlorophyll content and CO_2 gas exchange in barley leaves. Physiol Plant. 1992;85(4):632-8. https://doi.org/10.1111/j.1399-3054.1992.tb04765.x

24. Cutolo EA, Guardini Z, Dall'Osto L, Bassi R. A paler shade of green: engineering cellular chlorophyll content to enhance photosynthesis in crowded environments. New Phytol. 2023;239(5):1567-83. https://doi.org/10.1111/nph.19064

25. Eugenio da Silva A, de Souza Ferraz RL, Paulo da Silva J, da Silva Costa P, Viegas PRA, de Brito Neto JF, et al. Microclimate changes, photomorphogenesis and water consumption of 'Moringa oleifera' cuttings under different light spectrums and exogenous phytohormone concentrations. Aust J Crop Sci. 2020;14(5):751-60.

26. Vieira G. Gap dynamics in managed Amazonian forest: structural and ecophysiological aspects [thesis]. Oxford: University of Oxford; 1996.

27. Pattaro MC. The influence of blue light on the morphology of lettuce plants grown under different light qualities and intensities [dissertation]. Maringá: State University of Maringá; 2019.

28. Mariz-Ponte N, Martins S, Gonçalves A, Correia CM, Ribeiro C, Dias MC, et al. O potencial uso de UV-A e UV-B para melhorar a qualidade e preferência do tomate pelos consumidores. Sci Hortic. 2019;246:777-84. https://doi.org/10.1016/j.scienta.2018.11.058

29. Schulze PSC, Barreira LA, Pereira HGC, Perales JA, Varela JC. Light emitting diodes applied to microalgal production. Trends Biotechnol. 2014;32(8):422-30. http://dx.doi.org/10.1016/j.tibtech.2014.06.001

30. Lazzarini LES, Pacheco F, Silva ST, Coelho AD, Medeiros APR, Bertolucci S, et al. Utilização de diodo emissor de luz (LED) na fisiologia de plantas cultivadas – revisão. Sci Agrar Parana. 2017;16(2):137-44.

31. Landi M, Zivcak M, Sytar O, Brestic M, Allakhverdiev SI. Plasticity of photosynthetic processes and secondary metabolites under monochromatic light: a review. Biochim Biophys Acta Bioenerg. 2020;1861(1):148131.

32. Marani IKH, Rosa M, Silva FB, Alves NVS, Oliveira NR, Cavalcante WSS. Avaliação da utilização de diodos emissores de luz (LEDs) sobre as características morfofisiológicas de plantas de *Alternanthera brasiliana* Kuntze. In: Anais do XVI Congresso de Iniciação Científica da Universidade de Rio Verde; 2022; Rio Verde. Rio Verde: CICURV; 2022. 16(1). https://doi.org/10.29327/1244474.16-88

33. Centofante AR. Light quality on the morphoanatomy and physiology of Campomanesia pubescens seedlings. Sci Hortic. 2020;259:108761.

34. Yokoyama AH, Ribeiro RH, Balbinot Júnior AA, Franchini JC, Debiasi H, Zucareli C. Índice de área foliar e SPAD da soja após a safrinha e adubação nitrogenada e sua relação com a produtividade de grãos. Rev Ciênc Agrár. 2018;41(4):953-62. https://doi.org/10.19084/RCA18153

35. Shin YS, Lee MJ, Lee ES, Ahn JH, Kim MK, Lee JE, et al. Efeito do tratamento com diodos emissores de luz no crescimento e na qualidade da alface (Lactuca sativa L. 'Oak Leaf'). J Life Sci. 2014;24(2):148-53. https://doi.org/10.5352/JLS.2014.24.2.148

36. Kobayashi K, Amore T, Lazaro M. Diodos emissores de luz (LEDs) para alface hidropônica em miniatura. Optics and Photonics Journal. 2013;3(1):74-7.

37. Ebrahimi P, Shokramraji Z, Tavakkoli S, Mihaylova D, Lante A. Chlorophylls as natural bioactive compounds in food by-products: a critical review. Plants. 2023;12(7):1533. https://doi.org/10.3390/plants12071533