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The roles of light in a plant factory: photosynthesis efficiency and gas exchange parameters of lettuce as a function of light spectra

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ABSTRACT

Artificial light source is one of the most important factors for high quality and quantity vegetable production in a plant factory. Aiming to investigate the role of light spectra on growth, chlorophyll fluorescence, photosynthesis and stomata parameters in lettuce plants grown in a plant factory, a factorial experiment was conducted based on a completely randomized design with two lettuce cultivars (Lollo Rossa and Lollo Bionda) and four spectra LED illumination red (656 nm), red/blue (3:1) (656 nm), blue (450 nm) and white (449 nm). The results showed that the combination of red and blue LED light had the greatest effect on stomatal conductance (*gs*), number of stomata, length and width of stomata in both lettuce cultivars. On the other hand, the maximum substomatal CO_2 concentration (*Ci*) was observed in both lettuce cultivars when they were treated with red LED light. The results also showed that the maximum CO_2 assimilation rate (*PN*) was observed in Rossa variety under white LED and in Bionda cultivar under blue LED light. Contrary to the results related to some of photosynthetic parameters, the highest values of vegetative traits (plant height, dry and fresh mass of shoots and roots, leaf number and leaf area) of plants were observed in the treatment of red and blue light combination. It is concluded that plant growth, chlorophyll fluorescence characteristics, photosynthetic and stomatal properties can be affected by different spectra and cultivars of lettuce, so that the choice of proper lighting is a fundamental requirement for the cultivation of this plant.

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1. Introduction

The crucial factors that strongly influence the bio-synthesis and accumulation of various secondary plant compounds in lettuce are the quality, intensity and duration of light quality (Yang *et al*., 2022). Artificial lighting LED is a good light source for plant growth in plant factory, because this light source can regulate the light quality and quantity; thus, the use of this light source provides the necessary light for overall plant growth and development (Han *et al*., 2017). In addition, the light can improve the morphology of plants to numerous biotic and abiotic stresses (Meng *et al*., 2019). LEDs produce light with emission wavelengths, high photoelectric efficiency, photon flux or irradiance, and low heat generation. The use of LED lamps is one of the non-thermal food processing methods, with wavelengths ranging from 200 to 780 nm (Prasad *et al*., 2020). Light also plays an important role in photosynthetic apparatus and stomatal conductivity, so light deficiency and excess negatively affect photosynthetic parameters and stomatal conductivity level. Thus, bright light and low CO_2 concentrations stimulate stomata opening, while high CO_2 concentration, even in bright light, leads to stomata closing (Raschke, 1975). The use of LED lamps with the amount of radiation adapted to the needs of individual plants can cause the photosynthetic efficiency and increase of yield or biomass, especially in leafy vegetables (Kim *et al*., 2004). Meng *et al*. (2019) reported that treatment of lettuce plants with combination of red/blue (7R: 3B) light-emitting diodes had no negative effects on gas exchange and quantum yield but this light quality promoted the number of leaves, leaf width, number of roots, superoxide dismutase, and catalase activities, chlorophyll synthesis, and the accumulation of Chl *a* and Chl *b* and led to the highest fluorescence parameters. Muneer *et al*. (2014) indicated that high-intensity blue LEDs promote plant growth by controlling the integrity of chloroplast proteins that optimize photosynthetic performance in the natural environment.

The composite family (Asteraceae) is one of the largest families of flowering plants, with over 1600 genera and 2500 species worldwide. Some of its best-known taxa include lettuce, chicory, artichoke, daisies, and dandelions. Members of the Asteraceae have been used in food and medicine for centuries (Rolnik and Olas, 2021). Lettuce (*Lactuca sativa* L.) is one of the most widely consumed leafy vegetable crops worldwide. In 2018, the annual global production of lettuce was 27.6 million tons, and the top five producers were China, the United States of America, India, Spain, and Italy (Pérez-Urrestarazu *et al*., 2019). Lettuce leaves contain all recommended macro- and micronutrients for a healthy diet (Mou, 2012). Lettuce is low in calories and rich in fiber, vitamin C, and minerals, especially iron (Kim *et al*., 2016). Scientists also use lettuce as a reference species for the development of plant factories and, in general, for biological studies of vegetables in closed soilless systems (Miras-Moreno *et al*., 2020).

Today, water is a crucial natural resource, a basic human need, and a valuable natural resource (Zhushi Etemi *et al*., 2020). Agriculture, as the largest water user, is the most affected when a drought disaster occurs (Łabędzki and Bogdan, 2017). In this situation, scientists are looking for solutions to reduce water consumption in agriculture. One of them is growing plants hydroponically, because the benefits of hydroponics are numerous. In addition to higher yields and better water efficiency, hydroponic systems, when operated in a controlled environment, can be designed to provide continuous production throughout the year (Barbosa *et al*., 2015). Hydroponic systems are also used to grow various crops and specialty crops such as tomatoes, cucumbers, peppers, eggplant, strawberries, lettuce, and many others (Barbosa *et al*., 2015). Floating raft systems are one of the hydroponic systems for the production of vegetable crops, especially lettuce. Moreover, this system allowed the production of vegetables with high quality and quantity (Pantanella *et al*., 2010). In this method of hydroponic system, lettuce plants floating on styrofoam plates in a pond with nutrient solution grew well (Hussain *et al*., 2014; Silva *et al*., 2015). In this method, waste and loss of nutrient solution were reduced because the nutrient solution was prepared based on the nutrient needs of the plant.

Nowadays, chlorophyll fluorescence is proposed as a measurement criterion to measure the effects of environmental stress and to determine the degree of stress resistance (Strasser *et al*., 2004). Zheng and Van labeke (2017) suggested that 100% blue light and 75% red light with 25% blue light had the greatest effect on maximum quantum yield (F_v/F_m) and quantum efficiency in ornamental plant species. Light also has a great influence on plant growth and development. In addition, light as a source of energy can affect photosynthesis and related parameters, as well as the opening and closing of stomata. Photosynthesis affects several traits, and these parameters are influenced by light quality, with blue and red light playing the most important roles (Zheng and Van labeke, 2017). Photosynthetic parameters (especially net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), and intercellular CO_2 concentration (C_i)) respond differently to different light spectra (Roni *et al*., 2017). Hogewoning *et al*. (2010) reported that increasing blue light increases P_N and *gs* in cucumber plants. On the other hand, blue light affects components of stomatal function that appears to have a direct role in photosynthetic performance. The causes of the increase in photosynthesis under the influence of blue light treatment include a higher ratio of chlorophyll a and b (Leong and Anderson, 1984) and a higher content of ribulose-1,5-bisphosphate carboxylase/oxygenase (Ru-BisCO) (Eskins *et al*., 1991). Also researchers indicated that 100% blue light increased stomatal conductance that was associated with an increase in stomatal index and stomatal density (Zheng and Van Labeke, 2017). Although , a re-duction in blue light during the growth phase of lettuce decreased the photosynthetic rate more than red light, but the application of more than 10% blue light to lettuce plants increased the photosynthetic rate (Son and Oh, 2015; Brechner and Both, 2014). Roni *et al*. (2017) found that Eustoma leaves grown under blue light had higher net photosynthetic rates (P_N), stomatal conductance (g_s), and transpiration rates (E). Muneer *et al*. (2014) found that the use of high-intensity blue LED light promoted plant growth in lettuce plants by controlling the integrity of chloroplast proteins that optimize photosynthetic performance in the natural environment.

Researchers found that an increased ratio of red to blue light significantly increased the growth parameters of lettuce (*Lactuca sativa* L. var Lollo rosso), but a high proportion of blue light increased photosynthetic parameters and pigment content at different growth stages

(Azad *et al*., 2020). In another study, growth parameters such as leaf area, fresh and dry mass, and leaf optical indices such as chlorophyll and carotenoids were found to elicit a species-dependent response under the combination of red and blue LED light (Brazaitytė *et al*., 2021). Borowski *et al*. (2015) found that the combination of red and blue LED light increased the yield of lettuce plant in plant factory. The results of the study on 'Elizium' romaine lettuce showed that the combination of red and blue LED light increased the vegetative characteristics (Matysiak *et al*., 2021). Red light was reported to significantly increase shoot and leaf biomass, plant height, number of leaves per plant and stem diameter by increasing Chl content, thus promoting the highest photosynthetic capacity in ramie (*Boehmeria nivea* L.) (Rehman *et al*., 2020). Also, Oh *et al* (2021) reported that the fresh and dry mass of shoots and roots in *Rubus hongnoensis* was highest under R and RGB light. Other studies found that red light was most important for biomass production and chlorophyll *b* content of *Brassica Juncea*, while blue light was preferable for Chlorophyll a (Afridi *et al*., 2020). Although chen *et al* (2021) reported that red light increased growth parameters and photosynthetic pigments such as chlorophyll *a*, *b* and total chlorophyll and carotenoids in lettuce plants. Dyśko and Kaniszewski (2021) indicated that the use of LED light in cucumber and tomato increased vegetative parameters as well as total and marketable yields

In this study, we used a replacement nutrient solution based on the needs of the plants, based on previous experience with tomatoes (Gent and Short, 2010) and lettuce and basil plants (Domingues *et al*., 2012; Trejo-Téllez *et al*., 2012; Mattson and Peters, 2014; Gent, 2017; Van der Lugt *et al*., 2020) in a closed hydroponic system. The advantages of this method of plant feeding are better water and nutrient management, lower water and nutrient consumption, lower environmental impact, and higher yield per unit area. In the replacement treatment (invented by the author) based on the needs of the plant, obtained from the process of element uptake by the lettuce plant based on scientific sources (Gent and Short, 2010, Gent, 2017; Ronzoni, 2017); potassium nitrate was used completely in the same concentration as in the original solution, but the consumption of calcium nitrate, magnesium sulfate and potassium dihydrogen phosphate was reduced by three quarters and the microelements (iron, zinc, copper and manganese) were reduced by half and added every two days based on the volume of water added to the plastic container in which the plants were cultivated. For example, if the lettuce plant took up one liter of the solution in two days, one liter of distilled water was first added to the plant's container to compensate. The amount of concentrated solution of various fertilizers required for this one liter was determined based on the original formula, and concentrated solutions in the ratio given above were used instead of the complete ones. The reason for this was to prevent the accumulation of elements such as magnesium, calcium, phosphorus, and even microelements in the solution and to avoid their toxicity to the plant, since their absorption rate, according to the sources, is lower than that of potassium and nitrate. In this study, the concentrations of nutrients such as nitrogen (Kjeldahl), calcium and magnesium (by titration), potassium and sodium (flame photometer), phosphorus (spectrophotometer), iron, manganese, copper and zinc (atomic absorption) (Estefan *et al*., 2013) were measured weekly in the solution used for this study. Every year, greenhouse keepers inject millions of liters of water and nutrient elements into the environment after each use for plants, which causes severe damage to the environment and, on the other hand, wastes a large amount of water and nutrient elements. According to the mentioned cases, the purpose of this experiment is to increase the vegetative and physiological characteristics of lettuce varieties (Lollo Rossa and Lollo Bionda) in floating culture with the least amount of water and nutrients. Also, we intend to increase the morpho-physiological characteristics of lettuce plants grown in a factory plants under floating culture conditions by manipulating the light spectrums.

2. Materials and Methods

2.1.Plant material and growth conditions.

This experiment was conducted in the plant experiment factory of Vali-e-Asr University in 2020. The seeds of two lettuce cultivars (Lollo Rossa and Lollo Bionda) were obtained from Sepahan Rooyesh Isfahan and Rijk Zwaan Co. Lettuce seeds were planted in a seed tray filled with perlites. After the four-leaf stage, the seedlings were repotted into a small plastic pot with perlite. These small pots were placed in the holes of the floating systems. After transferring the plants to the floating systems, the Resh nutrient solution formulated for lettuce (Resh, 2022) (EC: 1.2 ds m^{-1}, pH: 6.5) was used (Table 1). The floating system consisted of 24 square plastic containers measuring 25 × 30 × 30 cm, each floating on a Styrofoam measuring 5 × 30 × 30 cm, and four plants planted in each container. All culture containers were connected to the air pump (HAILA, model: ACO-388 D) through holes and the nutrient solution was aerated for 24 hours. Every second day, the consumption of the nutrient solution was measured. Then, the plastic container was refilled with a modified Resh nutrient solution according to the nutrient needs of the plant during the growing period. Plants were grown in the plant factory at a temperature of 25/15 (day/night), a photoperiod of 12/12 hours (day/night), and a relative humidity of 50 ± 10%.

Table 1 The concentration of Resh nutrient solution (Resh, 2022) was used in this experiment.

Macronutrients	Concentration (mg L^{-1})	Micronutrients	Concentration (mg L^{-1})
N	139	Fe	0.94
P	31	Mn	0.14
K	215	Zn	0.13
Ca	84	B	0.16
Mg	24	Cu	0.03
S	35	Mo	0.03

2.2. LED tubes and the light treatments.

Lettuce plant cultivated under LED lamps with 24W of power (Parto Roshd Novin Company, Iran) of different spectral ranges: red (R, with peak 656 nm), red/blue (3:1) (R: B, with peak 656 nm), blue (B, with peak 450 nm) and white (W, with peak 449 nm). Photon flux density (PPFD) was 215 ± 5 µmol m^{-2} s^{-1} in all treatments (Fig. 1 and Table 2). The photoperiod of 12 hours was maintained. The LED light systems were mounted 30 cm above each of the plants separately.

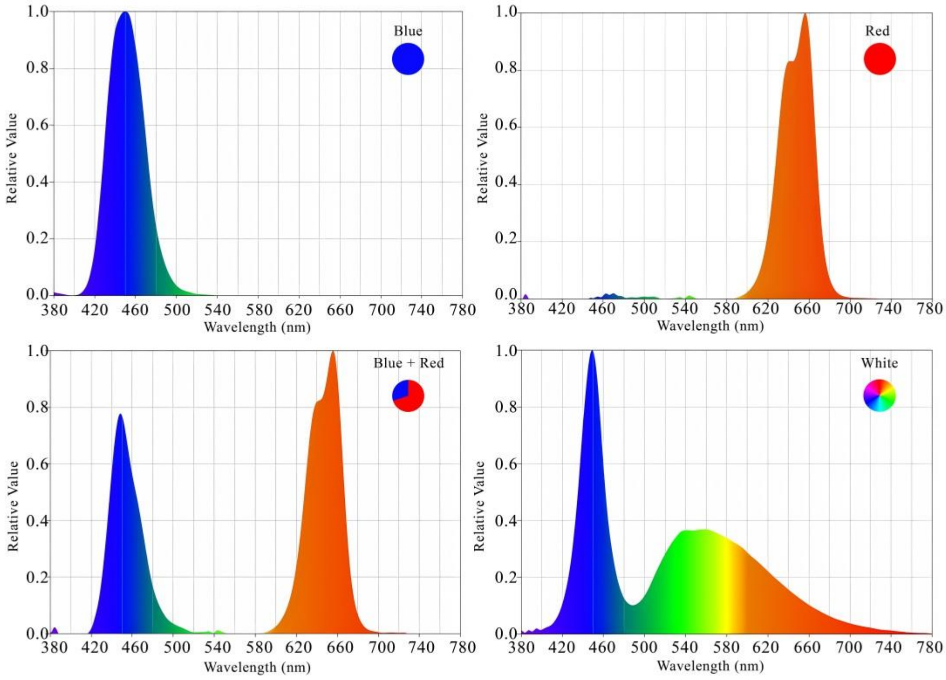


Figure 2 Relative distribution of different spectral LEDs (red, red/blue (3:1), blue and white) used during plant growth

Table 2 characteristics of LEDs used in this experiment

Manufacture Company	Efficiency	N. of LEDs	Light coverage area	Power consumption	Lens type	Input Voltage	DC Voltage	Output Current	Output Frequency
Iran Grow Light	90%	24	40cm × 100cm	24×3W	90*	AC100-260V	54-84V	600mA± 5%	50/60Hz

2.3. Vegetative characteristics

At the end of the experiment (40 days after transplanting), the plants were harvested from each plastic container and divided into shoots and roots. Dry mass (DM) was obtained when samples were dried in an oven for 72 h at 70 ^{o}C. The number of green leaves and leaf area (with Leaf area meter, CI-202, USA) and stem diameter (with caliper) for each treatment was recorded in the end of the experiment.

2.4. Yield index

To measure the yield index, at the end of the experiment, the plants will be separated from the crown area and then the mass of each plant will be calculated by a digital scale. According to the number of plants per unit area and the total mass of plants in each treatment, the biomass yield (head mass) per unit area will be determined and finally the yield index will be calculated based on the mass of the plant per square meter.

2.5. Measurement of photosynthetic pigments

The amount of chlorophyll a and b and total chlorophyll was measured using Porra's method (1989) with random sampling of 0.25 grams of leaf mass of three mature leaves of each plant and three replication and extraction with 80% acetone and centrifuged at 350 rpm for 10 minutes and then the samples was read using a spectrophotometer at wavelengths of 646.6 and 663.6 nm. Total carotenoid measurement Carotenoids were used according to the method of Lightenthaler (1987) and the absorbance was read at the wavelength of 470 nm.

$$\text{Total Chl} = [(76.17 \times OD_{646.6}) + (7.34 \times OD_{663.6})] \times [V/W]$$

Chl a= [(12.25 × $OD_{663.6}$) - (2/22 × $OD_{646.6}$)] × [V/W]
Chl b = [($OD_{646.6}$ × 20.31) - ($OD_{663.9}$ × 91.4)] × [V/W]
Car= (1000 OD_{470} - 3.27 [chl *a*] - 104 [chl *b*])/227)
OD: read absorbance V: the volume of acetone used W: sample mass (grams)

2.6. Evaluation of Chlorophyll Fluorescence

Chlorophyll (Chl) fluorescence was measured 45 days after LED lamps application using the portable photosynthetic efficiency analyzer (PEA, Hansatech Instruments Ltd., UK). After samples (fully developed leaves) dark adaptation for 15 min, Chl fluorescence parameters were measured and further ones were calculated (Table 3) with the help of the 'PEA Plus' software package (version 1.02). Basic Chl fluorescence parameters derived from the fluorescence parameters were as follows Brestič and Živčák (2013).

Table 3 Chl fluorescence parameters

Basic parameters derived from the extracted data		
F_0	Minimal fluorescence yield of the dark-adapted state	F_0 = F50µs
F_m	Maximal fluorescence yield of the dark-adapted state	$F_m = F_p$
F_v	Variable fluorescence	$F_v = F_m – F_0$
F_v/F_m	Maximal quantum yield of PSII photochemistry	$F_v/F_m = 1– (F_0/F_m)$
V_J	Relative variable fluorescence at time 2 ms (J-step) after the start of an actinic light pulse	$V_J = (F2ms – F_0)/(F_m – F_0)$
V_I	Relative variable fluorescence at time 30 ms (I-step) after the start of an actinic light pulse	$V_I = (F30ms – F_0)/(F_m – F_0)$
Area	The area above the OJIP curve; expresses the size of the reduced PQ pool	
Quantum yields		
ΨE0	The probability that a trapped exaction moves an electron into the electron transport chain beyond	QA $\Psi_0 = (1 – V_J)$
ΦD0	Quantum yield of energy dissipation	$\Phi D_o = F_0/F_V$
φET	Quantum yield of electron transport from QA to QB in PSII	$\varphi ET20 = \Phi P_o(1 – V_J)$
φRE	Quantum yield of reduction of end electron acceptors at the PSI acceptor side	$\varphi RE10 = \Phi P_o(1 – V_I)$
PI_{abs}	The performance index for the photochemical activity (basic formula on absorption basis)	$PI_{abs} = [1– (F_0/F_m)]/(M_0/V_J) \times (F_m – F_0)/F_0 \times (1 – V_J)/V_J$
PI_{total}	The total performance index for the photochemical activity (including the flow beyond PSI)	$PI_{tot} = PI_{abs}(1 – V_I)/(V_I – V_J)$
Specific energy fluxes (per QA-reducing PSII reaction center)		
TR0/RC	Trapped energy flux per RC (at t = 0)	$M_0(1/V_J)$
ET0/RC	Electron transport flux per RC (at t = 0)	$M_0(1/V_J)\psi_0$
Leaf Gas Exchange		
P_N	Net photosynthetic rate (µmol m^{-2} mol^{-1})	
E	Transpiration rate (mol (H_2O) m^{-2} s^{-1})	
g_s	Stomatal conductance (mol H_2O m^{-2} s^{-1})	
C_i	Intercellular CO_2 concentration (µmol (CO_2) mol^{-1})	
P_N/C_i	Instantaneous carboxylation efficiency	
WUE_i	Intrinsic water-use efficiency (P_N/gs) (µmol CO_2 mol H_2O^{-1})	

2.7. Stomata Characteristics and Leaf Gas Exchange

Samples were collected from the three fully expanded second leaves of two plants at the same position for each treatment. For the measurement of stomata parameters (width, length, and density), transparent nail polish was smeared on the surface of the leaves. The slides of three plant of each three replication made by the leaf epidermal fingerprint with the transplant nail polish method Zheng *et al*. (2013) were observed by optical microscope (Olympus Inc., Japan). The length, width, and density of stomata were measured with Image-tools software.

To measure photosynthesis traits were used by IRGA (LCi Ultra Compact, ADC BioScientific Ltd, Herts, UK). For measurement, three adult leaves from each plant in each container were selected from each replicate and placed in a special holder for the device, after six minutes, the relevant parameters were recorded. Instantaneous carboxylation efficiency (mmol/m^2) was obtained by dividing photosynthesis by Sub-stomatal CO_2 concentration. To determine the instantaneous intrinsic water-use efficiency (micromoles of CO_2 per mol of H_2O), the amount of photosynthesis was divided into stomata conductance (Ritchie *et al*., 1990).

2.8. Statistical Calculations

The experiment was a complete randomized design with two factors in three replications in factorial form and two plants of each variety in plastic container. Using SAS software version 9.4 to analyze all data (SA stitute, Cary, NC, USA). All data were statistically analyzed using two-way ANOVA model.When analysis of variance (ANOVA) indicated significant treatment effects, significant mean differences ($P<0.05$) were calculated by the LSD Multiple Range Test as a post hoc. Once the differences between the means are demonstrated, it is possible to determine which means are different using post hoc range tests and pairwise multiple comparisons. Range tests identify homogeneous subsets of means that do not differ from each other. The chlorophyll fluorescence parameters were calculated using the software "PEA Plus" version 1.12 (Hansatech). A correlation plot was drawn with Origin Pro software version 2021. The graphs were made using Excel 2013 (Microsoft, Redmont, WA, USA). The results were expressed as mean values and their standard errors (SE) using MS Excel software.

3. Results

3.1. Vegetative parameters and yield index

The results showed that vegetative parameters, especially fresh and dry mass of shoots, fresh and dry mass of roots, leaf area, number of leaves, leaf width, plant height and stem diameter were influenced by different LED quality, lettuce varieties and their interactions (Table 4), but leaf length was influenced only by light quality. Yield index was also affected by LED quality and the interactions between LED quality and lettuce cultivars, but lettuce cultivar alone did not affect this trait (Table 4). The results of this study showed that using the combination of red and blue LED in the Lollo Rossa cultivar increased the fresh and dry mass of the aboveground parts, but in the Lollo Bionda cultivar, the combination of red/blue and white LED light increased these traits compared to the other two LED light qualities (Table 5).

In this study, the fresh mass of the roots increased in the variety Lollo Rossa under the influence of the combination of red and blue light, which showed no statistically significant difference in the variety Lollo Bionda to the light qualities red, combination of red/blue and white LED (Table 5). The results also showed that the use of the combination of red and blue light resulted in an increase in root dry mass of the variety Lollo Bionda compared to the other interactions used. In the Lollo Rossa cultivar, the combination of red and blue light increased the root dry mass compared to the other LED light treatments (Table 5).

The results of our tests showed that the leaf area of the variety Lollo Rossa increased with the combination of red and blue light LED compared to the control treatment (white light). Also in Lollo Bionda variety, the use of red light and the combination of red/blue LED light and white LED light resulted in higher values of leaf area compared to red and blue LED light (Table 5).

Also, the number of leaves increased in this study under the influence of the combination of red and blue LED in both lettuce varieties compared to white light (Table 5). In addition, the results of our study showed that the combination of red and blue LED light in Lollo Rossa cultivar and the combination of red and blue LED light and white LED light in Lollo Bionda cultivar increased leaf width compared to red and blue monochromatic light treatment (Table 5).

In the present study, white LED light had the greatest effect on plant height in the Lollo Rossa cultivar, but the highest plant height was observed in the Lollo Bionda cultivar with the combination of red and blue LED light (Table 5). Stem diameter increased in this study in the Lollo Rossa cultivar with the combination of red and blue LED light, but in the Lollo Bionda cultivar, the combination of red and blue light and red monochromic increased the stem diameter (Table 5).

The yield index of lettuce cultivars was affected by the combination of red and blue LED light in both lettuce cultivars compared to other light treatments, but in Lollo Bionda cultivar, no significant difference was observed between the combination of red/blue LED light and white LED light in terms of yield index. (Table 5).

3.2. Photosynthetic pigments

The results of this study showed that SPAD index and photosynthetic pigments were affected by different LED quality, lettuce varieties and their interactions (Table 6). The results also showed that using a combination of red and blue LED light increased spade index in lollo Bionda cultivar, also in lollo Rossa cultivar, treating plants with a combination of red and blue LED light increased spade index, but there was no significant difference with the combination of red/blue light, monochromatic red light and white LED light in the variety lollo Bionda with the spade index in the combination of red/blue in the variety lollo Rossa (Table 7).

The amount of chlorophyll *a*, *b*, total chlorophyll and caretonied was increased by the combination of red and blue LED and white LED light in the cultivar lollo bionda, but in the cultivar lollo rossa the combination of red/blue had the highest amount of chlorophyll a and b compared to other light treatments (Table 7).

3.3. Chlorophyll fluorescence parameters

The results showed that chlorophyll fluorescence parameters, especially F_0, F_m and area were affected by different LED quality, lettuce cultivars, and their interactions (Fig. 3A-F and Table 8). Also F_v, PI_{abs} and ΦET10 was affected with LED quality and interactions of LED quality and lettuce cultivars but lettuce cultivar alone did not effect on F_v, PI_{abs} and ΦET10.. The results also showed that the white LED spectra in Rossa lettuce cultivar and the red and blue LED light in Lollo Bionda had the highest F_o value, which was a significant difference from the other treatments (Fig. 3A). The results showed that the highest amount of F_m was observed in the Lollo Rossa cultivar treated with the white LED quality but other light quality in this cultivar had no statistically significant togather, furthermore the use of blue LED light in lollo Bionda was increased the amount of Fm compared to the other light qualities (Fig. 3B). The results of Chl fluorescence also showed that the interaction of white LED and Rossa variety and blue LED and Bionda variety had the highest F_v value (Fig. 3C). The highest area was observed in the interaction between the Bionda variety and the blue LED light (Fig. 3D). The interaction between the Bionda cultivar and the blue LED also showed the highest PI_{abs} value compared to the other treatments, and there were no significant differences between the LED spectra of the Rossa cultivar (Fig. 3E). Results also showed that the use of blue LED light on Lollo Rossa and combination of Red/Blue on Lollo Bionda increased amount of ΦET but other interactions between LED light and lettuce varieties had no significant effect on ΦET (Fig. 3F).

Table 4 ANOVA results of the effect of different light spectra and lettuce variety on growth parameters in lettuce.

Source of variations	df	Shoot Fersh Mass	Shoot Dry Mass	Root Fersh Mass	Root Dry Mass	Leaf Area	Number of Leaf	Leaf Length	Leaf Width	Plant Height	Stem Diameter	Yield index
Light (L)	3	19509**	7.04**	74.3**	2.98**	19600**	85.1**	30.0**	4.33**	31.06**	2.58*	45.20**
Variety (V)	1	4752**	4.6**	36.6**	0.011*	11932**	114.7**	0.62^{ns}	1.04**	4.27**	0.91**	0.15ns
L × V	3	6406**	2.7**	38.7**	0.52**	7991**	150.4**	3.8^{ns}	2.30**	19.11**	1.17**	18.96*
Error	16	93.6	0.05	5.33	0.002	19.7	2.18	3.69	0.014	0.074	0.02	5.97
CV%	-	3.88	3.55	12.62	1.68	1.30	6.5	18.7	2.17	1.71	3.95	19.06

**, * and ns–significant at P≥0.01 and p≤0.05 and non-significant, respectively.

Table 5 Effect of the interaction of different light spectra and variety on growth parameters of lettuce. Means followed by the same letter for a parameter are not significantly different according to the LSD (p ≤ 0.05).

Light Spectra	Variety	Shoot Fersh Mass (g)	Shoot Dry Mass (g)	Root Fersh Mass (g)	Root Dry Mass (g)	Leaf Area (cm^2 per plant)	Number of Leaf	Leaf Width (cm)	Plant Height (cm)	Stem Diameter (mm)	Yield Index (Kg/m^2)
red	Rossa	224.2±4.6d	6.2±0.05d	17.9±0.06b	2.3±0.02g	297.4±0.6f	18.3±0.8e	5.26±0.06d	15.5±0.06e	4.01±0.02c	12.5±0.26bc
Red/blue		353.8±3.2a	8.61±0.30a	23.9±0.34a	3.47±0.03b	441.3±0.8a	32.4±1.3a	7.27±0.08a	14.4±0.05f	5.54±0.04a	18.2±0.63a
white		196.4±1.7e	5.7±0.15e	17.2±0.18b	3.10±0.02c	313.0±0.4e	16.6±0.7ef	4.87±0.05e	20.9±0.09a	3.91±0.08c	11.2±0.48bc
blue		165.1±1.4f	4.52±0.14f	9.15±.16c	2.66±0.04e	220.0±0.5g	14.2±0.6f	3.86±0.04f	11.3±0.05g	2.90±0.09d	9.4±0.43c
red	Bionda	257.2±5.0c	6.7±0.21c	19.6±0.37ab	2.45±0.05f	385.0±0.7b	21.6±1.1d	5.66±0.07c	16.0±0.04d	4.54±0.15b	9.42±0.95c
Red/blue		294.9±3.2b	7.23±0.25b	19.9±0.35ab	4.31±0.03a	384.9±0.8b	28.9±0.7b	6.13±0.05b	17.4±0.04b	4.67±0.13b	16.9±0.8ab
white		294.3±2.9b	7.18±0.25b	20.4±0.38ab	2.87±0.02d	353.0±0.5c	22.4±0.6d	5.94±0.06b	16.8±0.05c	4.62±0.1b	14.7±0.7b
blue		205.8±1.8e	6.04±0.18de	18.0±0.25b	2.13±0.02h	327.2d±0.6	26.0±0.8c	5.20±0.07d	15.2±0.08e	4.09±0.09c	11.7±0.6bc

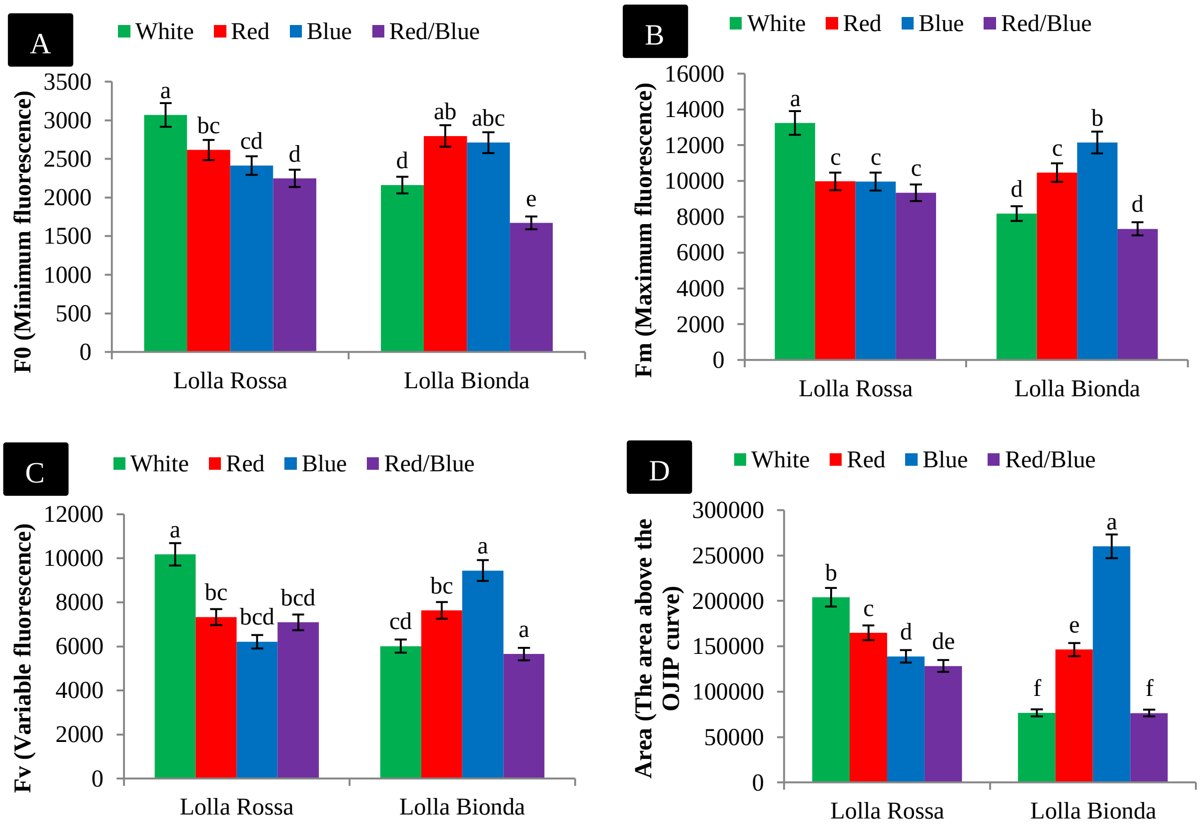
Table 6 ANOVA results of the effect of different light spectra and lettuce variety on photosynthesis and stomatal parameters in lettuce.

Source of variations	Df	SPAD Index	Chlorophyll *a*	Chlorophyll *b*	Total Chlorophyll	Carotenoid
Light (L)	3	1777.7**	0.095**	0.03**	0.26**	0.05**
Variety (V)	1	45.7**	0.023**	0.01**	0.12**	0.0005**
L × V	3	1178.4**	0.074**	0.01**	0.19**	0.03**
Error	16	1.79	0.0002	0.00007	0.001	0.00007
CV%	-	1.64	1.69	1.94	3.32	2.40

** and *–significant at P≥0.01 and p≤0.05 respectively.

Table 7 Effect of the interaction of different light spectra and variety on spad index and photosynthetic pigments of lettuce. Means followed by the same letter for a parameter are not significantly different according to the LSD (p ≤ 0.05).

Light Spectra	Variety	SPAD Index (%)	Chlorophyll *a* (mg/FM)	Chlorophyll *b* (mg/FM)	Total Chlorophyll (mg/FM)	Carotenoid (mg/FM)
red	Rossa	70.7±1.15e	0.74±0.05e	0.38±0.003de	1.08±0.006e	0.34±0.003d
Red/blue		84.4±0.85bc	0.86±0.04c	0.48±0.004b	1.34±0.004c	0.42±0.003c
white		70.1±.78e	0.75±0.02d	0.37±0.001e	1.09±0.005e	0.25±0.002g
blue		54.2±0.5f	0.59±0.01f	0.29±0.001f	0.85±0.002f	0.27±0.002f
red	Bionda	84.9±0.75bc	0.87±0.01c	0.46±0.005c	1.34±0.006c	0.31±0.003e
Red/blue		93.2±0.28a	1.13±0.02a	0.59±0.003a	1.73±0.005a	0.55±0.005a
white		86.5±1.25b	0.94±0.01b	0.48±0.002b	1.44±0.003b	0.46±0.004b
blue		76.4±1.29d	0.78±0.02d	0.39±0.004d	1.21±0.002d	0.26±0.002fg



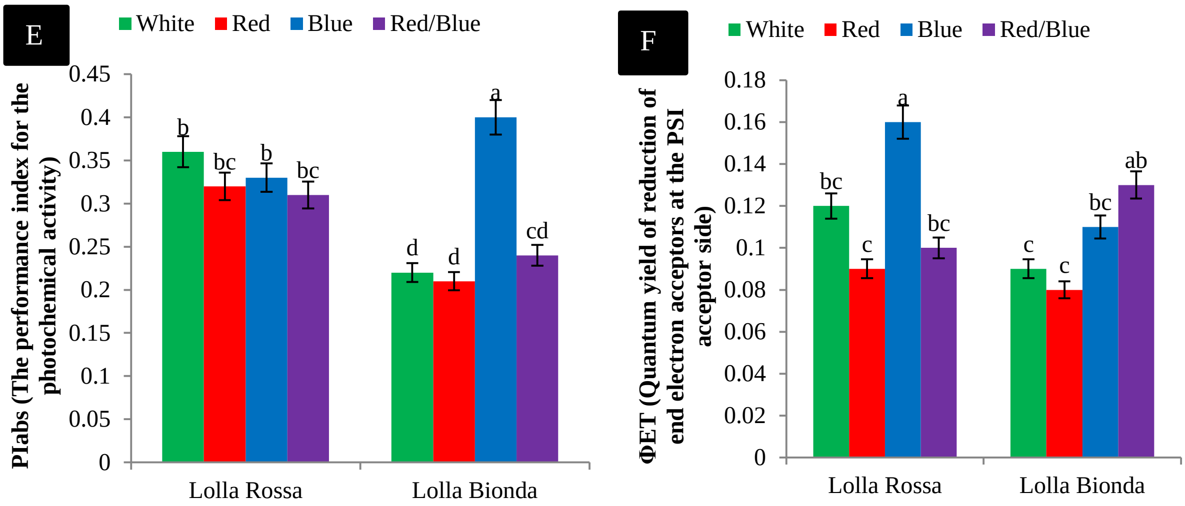


Figure 3 A-F The effect of different light spectra and varieties on F_0 (A), F_m (B), F_v (C), Area (D), PI_{abs} (E) and ΦET (F) of lettuce in the plant factory. Bars indicate standard error. Columns with different letters are significantly different at $P \leq 0.05$. F_0 – Minimal fluorescence yield of the dark-adapted state; F_m – Maximal fluorescence yield of the dark-adapted state; F_v –Variable fluorescence; Area - Area above the OJIP curve; it expresses the size of the reduced PQ pool; PI_{abs}- Performance index for the photochemical activity and ΦET- Quantum yield of reduction of end electron acceptors at the PSI acceptor side.

3.4. Leaf gas exchange

Analysis of ANOVA (Table 9) showed that different light spectra, lettuce varieties, and their interaction have a significant effect on photosynthesis. The highest amount of the internal CO_2 concentration (C_i) was observed under red LED and Rossa variety compared to other treatments (Table 10). The use of red/blue LED on the Bionda variety and on the Rossa variety caused the highest and lowest amount of transpiration from the surface of lettuce leaves respectively (Table 10). Results indicated that the combination of red and blue LEDs light in both varieties of lettuce had a significant effect on the stomatal conductance compared to other treatments, and the lowest amount of stomatal conductance was observed in the red, blue and white LED and Rossa variety (Table 10). The results also showed that (Table 10) the white LED in Rossa and blue LED in Bionda variety caused the highest amount of CO_2 assimilation rate against other treatments. Also, the lowest amount of CO_2 assimilation rate was observed with red LED spectrum in the Bionda variety.

The white LED spectrum in Rossa lettuce variety and blue LED spectrum in Bionda caused the maximum amount of intrinsic water-use efficiency against other treatments, but there was no statistical difference between them. Furthermore, the use of the combination of red and blue LEDs light in Rossa variety and red LED spectrum in the Bionda variety have the lowest amount of intrinsic water-use efficiency (Table 10). The white LED spectrum in Rossa lettuce variety and blue LED spectrum in Bionda caused the maximum amount of instantaneous carboxylation efficiency against other treatments. Furthermore, the minimum amount of instantaneous carboxylation efficiency was observed in both lettuce verities under red LED spectrum (Table 10).

Table 9 ANOVA results of the effect of different light spectra and lettuce variety on photosynthesis and stomatal parameters in lettuce.

Source of variations	Df	Photosynthesis parameters						Stomata parameters		
		C_i	E	gs	P_N	WUE_i	P_N/C_i	No. of stomata	Length	Width
Light (L)	3	371976**	0.68**	0.002**	72.12**	91.6**	0.0002**	700**	7.17**	3.97**
Variety (V)	1	31755**	1.17**	0.003**	38.25**	2.05**	0.0001**	600**	12.7**	3.72**
L × V	3	82417**	0.005*	0.00001**	26.34**	26.59**	0.0001**	500**	1.06**	0.25**
Error	16	291.1	0.001	0.00002	0.056	0.057	0.0000002	25	0.07	0.009
CV%	-	2.10	2.51	9.19	2.85	3.65	4.22	12.5	1.64	0.74

** and * –significant at P≥0.01 and p≤0.05, respectively.

Table 8 ANOVA results of the effect of different light spectra and variety on chlorophyll fluorescence parameters of lettuce plants in the plant factory

Source of variations	Df	F_0	F_m	F_v	F_v/F_m	Area	V_j	V_i	Φdo	ψ0	TR0/RC	ET0/RC	PI_{abs}	PI_{total}	φET	ΦET
Light (L)	3	**	**	*	ns	**	ns	ns	ns	ns	ns	ns	**	ns	ns	**
Variety (V)	1	**	**	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
L × V	3	**	**	**	ns	**	ns	ns	ns	ns	ns	ns	**	ns	ns	**
CV%	-	8.06	6.15	11.25	5.53	4.59	4.03	14.86	10.78	4.95	24.63	6.40	12.94	9.81	11.04	16.79

**, * and ns–significant at P≥0.01 and p≤0.05 and non-significant respectively.

Table 10. Effect of the interaction of different light spectra and variety on photosynthesis and stomatal parameters of lettuce. Net photosynthetic rate (P_N); Transpiration rate (E); Intrinsic water-use efficiency (WUE_i); Intercellular CO_2 concentration (C_i); Stomatal conductance (gs). Means followed by the same letter for a parameter are not significantly different according to the LSD ($p \leq 0.05$).

Light Spectra	Veriaty	C_i (µmol (CO_2) mol^{-1})	E (mol (H_2O) m^{-2} s^{-1})	gs (mol H_2O m^{-2} s^{-1})	P_N (µmol m^{-2} mol^{-1})	WUE_i (µmol CO_2 mol H_2O^{-1})	P_N/C_i	N. stomata	Stomata length (mm)	Stomata width (mm)
White	Rossa	476±1.52^{g}	0.88±0.008^{g}	0.03±0.003^{d}	13.09±0.15^{a}	0.027±0.0002^{a}	30±2.88^{d}	16.2±0.05^{e}	12.57±0.032^{d}	14.8±0.20^{a}
Red		1302±11.2^{a}	1.01±0.003^{f}	0.03±0.003^{d}	4.8±0.09^{e}	0.003±0.0001^{f}	50±2.88^{b}	18.4±0.10^{b}	13.7±0.07^{b}	4.7±0.11^{c}
Blue		809±0.57^{c}	1.04±0.02^{f}	0.04±0.0001^{d}	4.85±0.11^{e}	0.005±0.0001^{e}	30±2.88^{d}	16.9±0.06^{d}	12.31±0.02^{e}	4.6±0.08^{c}
Red/Blue		801±2.08^{c}	1.67±0.008^{b}	0.07±0.003^{b}	5.39±0.18^{d}	0.006±0.0002^{e}	70±2.88^{a}	19.4±0.26^{a}	14.04±0.09^{a}	3.21±0.10^{e}
White	Bionda	721±2.33^{e}	1.33±0.008^{e}	0.06±0.0001^{c}	12.36±0.18^{b}	0.017±0.0003^{c}	30±2.88^{d}	15.9±0.03^{ef}	11.74±0.06^{f}	9.27±0.19^{b}
Red		1031±22^{b}	1.53±0.02^{c}	0.06±0.003^{c}	4.2±0.09^{f}	0.004±0.0001^{f}	30±2.88^{d}	16.2±0.08^{e}	12.4±0.02^{e}	2.7±0.10^{f}
Blue		583±11.2^{f}	1.45±0.02^{d}	0.06±0.0001^{c}	13.01±0.08^{a}	0.022±0.0005^{b}	40±2.88^{c}	15.5±0.32^{f}	11.84±0.03^{f}	8.93±0.18^{b}
Red/Blue		761±4.93^{d}	2.06±0.03^{a}	0.1±0.005^{a}	8.66±0.12^{c}	0.011±0.0001^{d}	40±2.88^{c}	17.4±0.09^{c}	13.54±0.05^{c}	4.19±0.015^{d}

*Columns with different letters are significantly different at $P \leq 0.05$

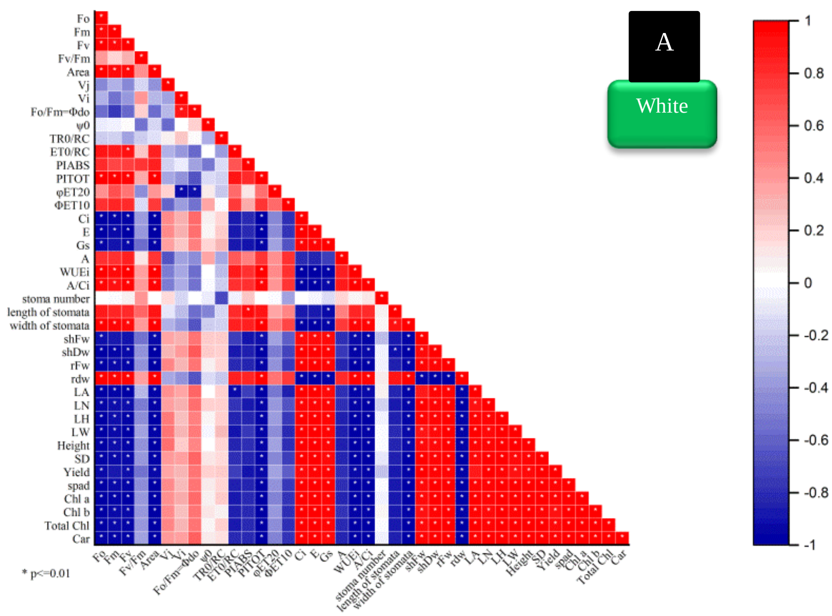
3.5. Correlation analysis

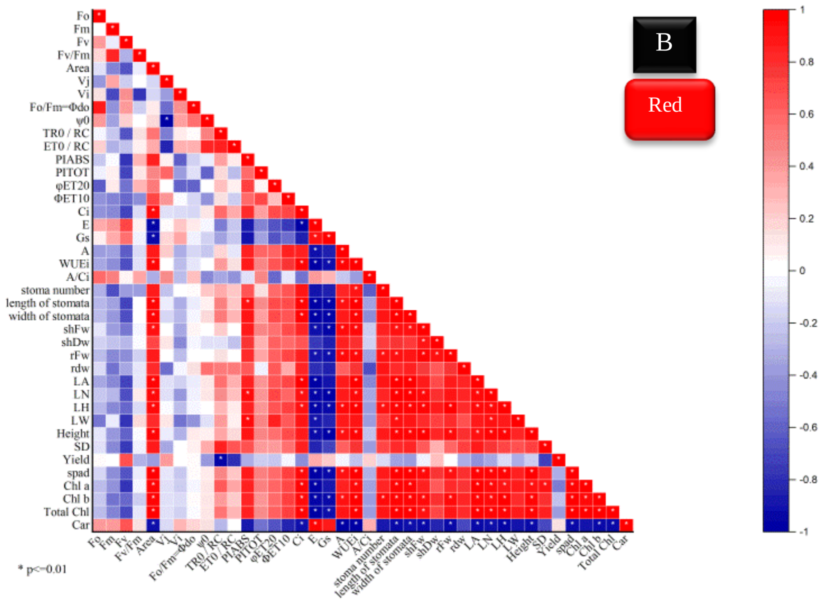
The correlation plot (Fig. 4A-D) shows the correlation between vegetative parameters, photosynthetic pigments, chlorophyll fluorescence, and gas exchange parameters of two lettuce cultivars. The results also show that under white LED light, a positive and significant correlation was observed between Ci, E and WUEi with growth parameters such as shoot fresh mass, root fresh mass, leaf number, leaf area, leaf height width and plant height, stem diameter, yield index and spad index and photosynthetic parameters (such as chlorophyll a, b and total chlorophyll and carotenoids). The results also show that vegetative parameters, chlorophyll a, b and total chlorophyll and carotenoids were positively correlated (Fig. 4A). The correlation of PN, WUHi and PN /Ci, stomata length and width with F0, Fm, Fv and area over OJIP curve was statistically significant and positive. (Fig. 4A).

The results also show that in red light LED area over OJIP curve is positively correlated with vegetative parameters, photosynthetic pigments (except carotenoids), and in this light quality PN and WUEi are positively correlated with stomata, vegetative parameters (except yield index), SPAD, Chlorophyll a, b, and total chlorophyll. In addition, the correlation of growth parameters (such as shoot and root fresh and dry mass, leaf number and leaf area, leaf height and width, and plant height) and chlorophyll pigments (such as chlorophyll a, b, and total chlorophyll) was statistically significant and positive (Fig. 4B) .

In this study, lettuce cultivars E, gs, P_N, WUH_i and P_N/C_i treated with blue LED light showed positive correlation with F_0, F_m, F_v, area, TRO/RC, PI_{total} and ΦET. The results also showed that C_i had a positive correlation with stomata length and number, growth parameters (except root dry mass and leaf height), and photosynthetic pigments (except carotenoid). The correlation of stomata number, length and width was positive with the growth parameters (except root dry mass and leaf height) and photosynthetic pigments (except cadaver formation). The results of growth parameters (except root dry mass) of lettuce palms and photosynthetic pigments (except carotenoids) were also statistically significant and positive (Fig. 4C).

Our results also showed that in the lettuce cultivar treated with a combination of red and blue spectra (in a ratio of 3:1), a high correlation was observed between F_0, F_m, Area, PI_{abs}, and *Ci* with stomata parameters, vegetative parameters (except root dry mass), and SPAD, chlorophyll a, b, and total chlorophyll and carotenoids. Also, for the quality of LED lamps, the correlation between stomata parameters and growth parameters (such as shoot fresh mass, root dry mass, leaf number and area, leaf height and width, and plant height except root dry mass) and chlorophyll pigments (such as chlorophyll *a*, *b*, and total chlorophyll and carotenoids) was statistically significant and positive (Fig. 4D).




B
Red
Fo
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Area
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PITOT
φET20
ΦET10
Ci
E
Gs
A
WUEi
A/Ci
stoma number
length of stomata
width of stomata
shFw
shDw
rFw
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Yield
spad
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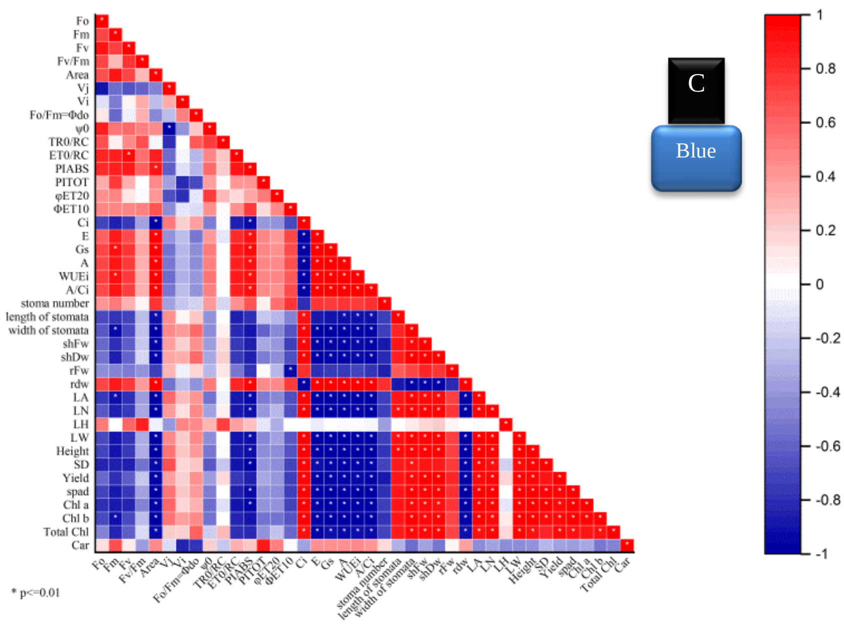
* p<=0.01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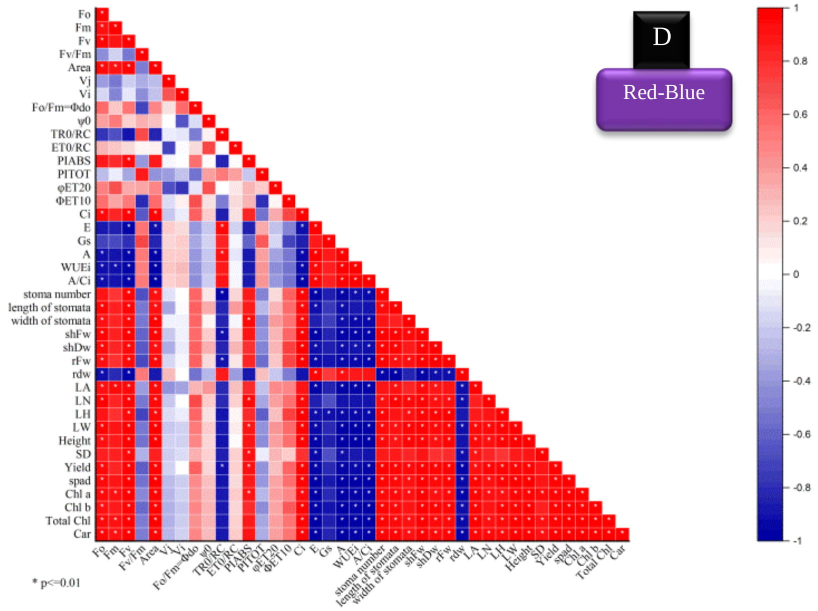

Figure 4 A-D. Correlation plot between growth parameters (shfw- shoot fresh mass; shdw- shoot dry mass, rfw- root fresh mass; rdw- root dry mass; LA- leaf area; LN- leaf number;LH- leaf hieght; LW -leaf width; SD- stem diameter; Hieght- plant hieght; Yield- yield index); SPAD- spad index; photosynthetic pigmants (Chl *a*- chlorophyll *a*; Chl *b*- chlorophyll *b*, Total Chl- total cholorophyll, Car- cartenoied), chlorophyll fluorescence parameters and gas exchange parameters (Net photosynthetic rate (P_N); Transpiration rate (*E*); Intrinsic water-use efficiency (WUE_i); Intercellular CO_2 concentration (C_i); Stomatal conductance (*gs*), Instantaneous carboxylation efficiency (P_N /C_i) of two lettuce cultivars treated with the different LED spectra (A- RED, B-Combination of Red and Blue, C- Blue and D- White spectra) in floating hydroponics. The size and color intensity of circles are proportional to Pearson's correlation coefficient at P < 0.01. Red circles indicate positive correlations, while blue are negative correlations. In the correlogram scale from − 1 to + 1, Pearson's correlation coefficient for variables is on the vertical and horizontal axis. *Indicates values that are statistically different at P < 0.01. A correlation plot was drawn with Origin Pro software version 2022b.

4. Discussion

Among the many environmental factors that affect plants, light is one of the most important factors affecting vegetative parameters. It has been found that different light qualities greatly alter the vegetative characteristics of plants, including leafy vegetables (Naznin *et al*., 2019). Light quality also stimulates signal transduction systems through different mechanisms to alter plant morphology, as light quality can regulate plant growth through different photoreceptors (Ward *et al*., 2005). Inhibition of plant height is regulated by the photoreceptor crypto-chromes and the maximum activity of this photoreceptor is stimulated by blue light (Ahmad *et al*., 1997; Ahmad *et al*., 2002). Researchers have shown that the combination of red and blue LED light increases the fresh mass and dry mass of shoots of lettuce, spinach, basil, kale and bell pepper (Naznin *et al*., 2019), lettuce (Wang *et al*., 2016) and eggplant and tomato (Wojciechowska *et al*., 2020), which is consistent with the results of this study. Also Zhang *et al* (2020) reported that the combination of these two spectrums improved the growth of *Salvia miltiorrhiza* more efficiently, For instance, the number of leaves, plant height and fresh mass of the whole plant. In this study, the combination of Red and Blue (70:30) stimulated plant height. In another study, Naznin *et al*. (2019) it was reported that different percentages of red and blue light had a significant effect on leaf number of spinach, basil and bell pepper, and these researchers also indicated that the effect of different percentages of R and B LEDs on leaf number depended on the plant species. In this study, leaf number was affected by the combination of red and blue

LED light, which is consistent with the results in spinach, basil and bell pepper (Naznin *et al*., 2019) and cucumber (Hernàndez *et al*., 2012). The results of our study also showed that the use of white LED light had a greater effect on vegetative traits of lettuce cultivars compared to red and blue monochromatic LED light. The increase in vegetative traits under the influence of the combination of red and blue LED light may be attributed to the role of these lights in photosynthetic traits and photosynthetic pigments (Bantis *et al*., 2018; Kang *et al*., 2016). Researchers also reported that the differences in vegetative traits under different light qualities may depend on the variety of lettuce plants (Frąszczak *et al*., 2021). A previous study showed that the combination of red and blue light LED (4R:1B) promoted the growth parameters, photosynthetic pigments, leaf structure characteristics and quality of spinach plant (Nguyen *et al*., 2021).

In the present study, the increase of photosynthetic pigments was observed under the influence of the combination of red and blue light. According to the results of lettuce and kale (Naznin *et al*., 2019), chili paper (Gangadhar *et al*., 2012), lettuce (Zhang *et al*., 2020) and lamb's lettuce (Wojciechowska *et al*., 2013), it was found that the combination of red and blue LED light increased the amount of chlorophyll a, b, total chlorophyll and carotenoids. Increasing the production and transfer of photosynthetic compounds is a factor for increasing the growth and development of plants, because red and blue light can promote the photosynthetic process, and blue light has the greatest effect on the production and transport of chlorophyll and the crucial catalyst to increase the carotenoid content in plant leaves (Hogewoning *et al*., 2010; Li *et al*., 2012; Briggs and Chirstie, 2002). In previous studies, the results showed that the combination of red and blue LED light increased the biomass of lettuce plant, resulting in higher Chl content and water and energy use efficiency (Pennisi *et al*., 2019). Also, in other studies, the combination of red and blue LED light increased photosynthetic performance and photosynthetic pigments (Hernández *et al*., 2016; Wang *et al*., 2015; Sabzalian *et al*., 2014). Fan *et al*. (2013) reported that the concentration of photosynthetic pigments and precursors of chlorophyll biosynthesis were higher under red/blue LED light in Chinese cabbage (*Brassica campestris* L.) without head. In this study, the results showed that the combination of red/blue LED light and white LED light increased photosynthetic pigments, especially chlorophyll a, b, total chlorophyll and carotenoid in lettuce cultivar Lollo Bionda. Nguyen *et al* (2021) reported that the combination of red/blue LED light (4R:1B) increased photosynthetic pigments in spinach plant.

In plants, chlorophyll fluorescence has been emitted as a sign of functioning photosynthetic machinery when abiotic and non-abiotic stress is applied to plants (Strasser *et al*., 2004). Chlorophyll (Chl) fluorescence techniques have been reported to be a very powerful tool for the non-destructive study of photochemical and non-photochemical processes in thylakoid membranes, chloroplasts, plant tissues, and whole plants (Roháček *et al*., 2008). In this experiment, blue LED light had more effect on the F_v, Area, and PI_{abs} and ΦET10 (Fig. 3 A-F), but the greatest Fv and Fm was absorbed in white LED light treatment (Fig. 3 A-F), which was different from the results of Meng *et al* (2019). The increase in Fv and Fm under white light treatment is Plants are most effective in carrying out the process of photosynthesis under white light, which includes all wavelengths. While plants take in all colors and wavelengths of light, some colors affect the plant species more than others (Williams *et al*., 2015). In addition, the use of monochromatic red light and the combination red/blue light spectrums could not have a significant effect on the parameters compared to white LED light. The use blue LED light treatment had a positive impact on the stimulation of PS I in the photosynthesis process which has been reported in *Cyanobacteria Bacteria* and *Arabidopsis thaliana* in previous research (Lamb *et al*., 2018). Also, in the current study, the significant photosynthesis activities under blue LED light treatment did not translate into an improvement in the growth rate of lettuce verities. Esmaeilizadeh *et al*. (2021) reported that the use of blue and white LED spectra had a positive effect on F_0, F_v, F_m, and Area in strawberry plants. Studies have shown that the use of different light qualities has a significant effect on chlorophyll fluorescence parameters in plants such as cucumber (Moazzeni *et al*., 2020). In another study, Meng *et al*. (2019) reported that the use of blue LED spectrum in gerbera decreased the amount of F_0, but when they were treated with red and combined blue and red light, the values of F_m and F_0 parameters increased. The results of Moazzeni *et al*. (Wang *et al*., 2021) also showed that cultivar *Sinningia speciosa* treated with a combination of red and blue LED light had the highest performance index of absorption energy flux. A significant decrease in F_0 and Fm was observed when the plants were treated by red and blue monochromic in Lollo Rossa and red monochoromic in Lollo Bionda lettuce varieties, which manifested as a smooth straight line in the kinetics of original Chl a fluorescence. The decreases in Fv with time in the kinetics of original Chl a fluorescence were attributed to stress that reduced the amount of chlorophyll in the leaf tissues, increased the dissipation of non radiative energy (DI0/RC), and rendered the PSII reaction center inactive (Tsai *et al*., 2019).

Our results in this experiment showed that, red LED light and the combination of red and blue LED spectrum had the greatest effects on *Ci*, *E*, and *gs*, stomata numbers, stomata widths and stomata lengths compared to white LED spectrum in both lettuce cultivars (Table 6). Also in the current research, the highest intrinsic water-use efficiency and net photosynthetic rate was observed in the treatment of white LED light. Therefore, we could suggest that the reduction in P_N under different light quality occurred perhaps due to the stomatal limitation, compared with the plants under W. It was also observed in acacia (Yu and Ong, 2003), and cucumber (Wang *et al*., 2009). Water use efficiency is defined as the amount of CO_2 taken up by plant photosynthesis relative to the amount of water lost through transpiration or evapotranspiration (Cernusak 2018). It can be determined using several methods according to the targeted scale for the study and the experimental design of the experiment (Zhang *et al*. 2023). Briefly, intrinsic water use efficiency (*WUEi*) considers ecophysiological processes driving CO_2 influx and water efflux without being sensitive to vapour pressure deficit (de Almeida Lobo *et al*. 2023). Therefore, this method, applicable on C3 plants, is particularly suitable for estimating *WUE* at the tree scale. Calculations of *WUEi* have recently been improved to embed the impact of mesophyll conductance on CO_2 diffusion in leaf cells (Stangl *et al*. 2019; Vernay *et al*. 2020). The results of this experiment show that the use of red and a combination of red and blue light-emitting diodes as light sources affects photosynthesis and stomata parameters may be by increasing chlorophyll absorption, which has also been reported by other researchers (Piovene *et al*., 2015; Kang *et al*., 2016). Of all the parameters affecting plant growth, light is an important environmental parameter affecting stomatal conductance (Wang *et al*., 2016; Kang *et al*., 2016). In addition, researchers reported that the combination of red and blue LED light is an effective and useful source for plant photosynthesis (Azad *et al*., 2020). It has been found that the low photosynthetic parameters of plants under red LED light were due to low nitrogen content in plant leaves, which is due to low chlorophyll and carotenoid content (Kim *et al*., 2004). Manipulation of LED spectra and especially the use of red and blue spectra (red peaks at 634 and 665 nm and blue peak at 451 nm) are more efficient for plant growth and photosynthesis (Roni *et al*., 2017). The combination of red and blue is used in commercial research and horticulture because these light wavelengths play an important role in photosynthesis and the efficiency of photosynthesis decreases if they are not used (Miao *et al*., 2017). Ahmad *et al*., (2016) reported that the use of red and blue light-emitting diodes increased the photosynthetic rate, transpiration rate, and stomatal conductance in lettuce plants. The researchers found that more than 90% of the blue and red light was absorbed by the plants. Blue light can have an indirect effect on stomata opening, and this effect is independent of photosynthetic activity (CO_2 decrease in the leaf); on the other hand, blue light can increase transpiration without having a significant effect on photosynthesis (Ahmed *et al*., 2022). Red light plays a role in stomata opening due to the response of stomatal guard cells to a decrease in intercellular CO_2 concentration and the direct response of chloroplast guard cells to red light (Matthews *et al*., 2020,; Shimazaki *et al*., 2007). Hogewoning *et al*. (2010) reported that blue light plays an essential role in chlorophyll biosynthesis; red light is also essential for this process. It is reported that the use of red light increases CO_2 assimilation rate, transpiration rate, and stomatal conductance. In addition, blue light, white light, and a combination of red and blue light were found to have the most significant effects on internal CO_2 concentration, intrinsic water use efficiency, and instantaneous carboxylation efficiency of strawberry plants (Miao *et al*., 2016). He *et al*., (2019) reported that combined red/blue spectra LED were more effective in promoting photosynthesis of lettuce than red LED or blue LED alone. Previous studies have shown that the use of blue light on lettuce plant had a positive effect on well-organised guard cells with open stomata and increased the number of stomata (Zheng *et al*., 2017). The results of our experiment showed that most open stomata and the number of stomata were observed under the combination of red and blue light (Table 6). In plants, stomata are important channels for the exchange of water and gases with external environmental conditions, so stomata conductance and proton driving forces are affected by light (He *et al*., 2019). Muneer *et al*. (2014) pointed out that one of the reasons for the reduction in transpiration rate and stomatal conductance in lettuce could be the closure of stomata with reduced normalised expression and number of stomata; also, in our study, the results showed that the use of a combination of red and blue light had the highest transpiration rate compared to other LED treatments in both lettuce cultivars. Based on results of our experiment, the growth parameters, photosynthetic pigments, gas exchange and stomatal characteristics had significant correlation under all used light spectrums (Fig. 4A-D). Stomatal movements control CO_2 uptake for photosynthesis and water loss through transpiration, and therefore play a key role in plant productivity and water use efficiency (Lawson and Vialet-Chabrand, 2019). Also, plant gas exchange and photosynthetic activity give insight into the energy balance of plants. These parameters, as well as transpiration, stomatal conductance, intercellular CO_2 concentrations, leaf chlorophyll and nitrogen contents, are especially interesting because they can be measured directly on the living organs (Parolin *et al*., 2010). The control of gaseous exchange between the leaf and bulk atmosphere by stomata governs photosynthetic CO_2 uptake for photosynthesis and transpiration, determining plant productivity and water use efficiency. The balance between these two processes depends on stomatal responses to environmental and internal cues and the synchrony of stomatal behavior relative to mesophyll demands for CO_2 (Lawson *et al*., 2014). Doheny-Adams *et al*. (2012) reported that the strong correlation between stomatal density and size was maintained within mutant plants: plants with lower stomatal densities also showed a greater mean stomatal size, whereas smaller stomata were found in leaves with greater stomatal densities. Van der Tol *et al*. (2009) reported a positive relationship between passively measured steady-state chlorophyll fluorescence and actual photosynthesis. In another study, it was found that significant link between net photosynthesis and steady-state fluorescence obtained under natural sunlight conditions (Zarco-Tejada *et al*., 2013).

5. Conclusions

Our work showed that lettuce plants treated with blue and white LEDs exhibited the highest values of photosynthetic efficiency, as evidenced by the increase in the values of F_0, F_v, F_m, Area, and PI_{abs} parameters in Rossa and Bionda cultivars. The results also showed that had the greatest effects on vegetative traits, *Ci*, *E*, and *gs*, stomata numbers, stomata widths and stomata lengths were observed in the treatment of red and blue light combination compared to white LED spectrum in both lettuce cultivars which shows the effect of this light spectrum on other growth effective factors in plants. Increasing the photosynthetic performance of plants by applying a particular light spectrum will be associated with poorer or better use of available water in the growth medium. This is a crop species dependent issue.

Declaration of Competing Interest

The authors report no declarations of interest.

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References

Afridi, M., Fayyaz, M., Rehman, M., Akhter, N., Masood, S., Khan, S. and Ahmad, K. 2020. Effect of Light Emitting Diode (LED) on the Growth and Photosynthetic Characteristics of Brassica Juncea. Journal of Bio-Molecular Sciences (JBMS), 7: 1-9.

Ahmad, M., and Cashmore, A.R. 1997. The blue-light receptor cryptochrome 1 shows functional dependence on phytochrome A of ohytochrome B in Arabidopsis thaliana. Plant Journal, 11: 421–427.

Ahmad, M., Grancher, N., Heil, M., Black, R.C., Giovani, B., Galland, P., and Lardemer, D. 2002. Action spectrum for cryptochrome-dependent hypocotyl growth inhibition in Arabidopsis. Plant Physiology, 129: 774–785.

Ahmed, H. A., Tong, Y., Li, L., Sahari, S. Q., Almogahed, A. M. and Cheng, R. 2022. Integrative Effects of CO2 Concentration, Illumination Intensity and Air Speed on the Growth, Gas Exchange and Light Use Efficiency of Lettuce Plants Grown under Artificial Lighting. Horticulturae, 8(3): 270.

Azad, M. O. K., Kjaer, K. H., Adnan, M., Naznin, M. T., Lim, J. D., Sung, I. J., and Lim, Y. S. 2020. The evaluation of growth performance, photosynthetic capacity, and primary and secondary metabolite content of leaf lettuce grown under limited irradiation of blue and red LED light in an urban plant factory. Agriculture, 10(2): 28.

Bantis, F., Smirnakoub, S., Ouzounisc, T., Koukounarasa, A., Ntagkase, N., and Radogloub, K. 2018. Current status and recent achievements in the field of horticulture with the use of light-emitting diodes (LEDs). Scientia Horticulturae, 235, 437–451.

Barbosa, G. L., F. D. Gadelha, N. Kublik, A. Proctor, L. Reichelm, E. Weissinger, G. M. Wohlleb. and R. U. Halden. 2015. Comparison of Land, Water, and Energy Requirements of Lettuce Grown Using

Hydroponic Vs. Conventional Agricultural Methods International Journal of Environmental Research and Public Health, 12: 6879-91.

Borowski, E., Michałek, S., Rubinowska, K., Hawrylak-Nowak, B., and Grudzinski, W. 2015. The effects of light quality on photosynthetic parameters and yield of lettuce plants. Acta Scientiarum Polonorum, 14(5): 177-188.

Brazaitytė, A., Miliauskienė, J., Vaštakaitė-Kairienė, V., Sutulienė, R., Laužikė, K., Duchovskis, P., and Małek, S. 2021. Effect of different ratios of blue and red LED light on Brassicaceae microgreens under a controlled environment. Plants, 10(4) 801.

Brechner, M., and Both, A. J. 2014. Cornell Controlled Environment Agriculture. Cornell University.

Brestic, M, and Zivcak, M. 2013. PS II Fluorescence Techniques for Measurement of Drought and High Temperature Stress Signal in Crop Plants: Protocols and Applications. In Molecular Stress Physiology of Plants, 87-131.

Briggs,W.R. and Christie, J.M. 2002. Phototropins 1 and 2: Versatile plant blue-light receptors. Trends Plant Science, 7, 204–210.

Cernusak, L.A. 2020. Gas exchange and water-use efficiency in plant canopies. Plant Biology, 22: 52-67.

Chen, X. L., Li, Y. L., Wang, L. C., and Guo, W. Z. 2021. Red and blue wavelengths affect the morphology, energy use efficiency and nutritional content of lettuce (*Lactuca sativa* L.). Scientific reports, 11(1): 1-12.

de Almeida Lobo, F., Previl, R., Gonzalez-Meler, M.A., Pereira, B.L.C., de Moura, L.C., Ortíz, C.E.R., da Cruz Genuncio, G. and Vourlitis, G.L. 2023. Is intrinsic water use efficiency independent of leaf-to-air vapor pressure deficit?. Theoretical and Experimental Plant Physiology, 35(2): 65-80.

Doheny-Adams, T., Hunt, L., Franks, P.J., Beerling, D.J. and Gray, J.E. 2012. Genetic Manipulation of Stomatal Density Influences Stomatal Size, Plant Growth and Tolerance to Restricted Water Supply across a Growth Carbon Dioxide Gradient. Philosophical Transactions of the Royal Society Biology Science, 367: 547-55.

Domingues, D.S., Takahashi, H.W., Camara, C.A. and Nixdorf, S.L. 2012. Automated system developed to control pH and concentration of nutrient solution evaluated in hydroponic lettuce production. Computers and Electronics in Agriculture, 84: 53-61.

Dyśko, J. and Kaniszewski, S. 2021. Effects of LED and HPS lighting on the growth, seedling morphology and yield of greenhouse tomatoes and cucumbers. HortScience, 48: 22–29.

Eskins, K., Jiang, C. Z., and Shibles, R. 1991. Light-quality and irradiance effects on pigments, light-harvesting proteins and Rubisco activity in a chlorophyll-and light-harvesting-deficient soybean mutant. Physiologia Plantarum, 83(1): 47-53.

Esmaeilizadeh, M., M. R. Malekzadeh Shamsabad, H. R. Roosta, P. Dąbrowski, M. Rapacz, A. Zieliński, J. Wróbel, and H. M. Kalaji. 2021. Manipulation of Light Spectrum Can Improve the Performance of Photosynthetic Apparatus of Strawberry Plants Growing under Salt and Alkalinity Stress. PLoS One, 16: 0261585.

Estefan, G., Sommer, R. and Ryan, J. 2013. Methods of soil, plant, and water analysis. A manual for the West Asia and North Africa region, 3: 65-119.

Fan, X., Zang, J., Xu, Z., Guo, S., Jiao, X., Liu, X. and Gao, Y. 2013. Effects of different light quality on growth, chlorophyll concentration and chlorophyll biosynthesis precursors of non-heading Chinese cabbage (*Brassica campestris* L.). Acta physiologiae plantarum, 35(9): 2721-2726.

Frąszczak, B, and Kula-Maximenko, M. 2021. The preferences of different cultivars of lettuce seedlings (Lactuca sativa L.) for the spectral composition of light. Agronomy, 11(6): 1211.

Gangadhar, B.H., Mishra, R.K., Pandian, G. and Park, S.W. 2012. Comparative study of color, pungency, and biochemical composition in chili pepper (*Capsicum annuum* L.) under different light-emitting diode treatments. HortScience, 47: 1729–1735.

Gent, M.P. 2017. Factors affecting relative growth rate of lettuce and spinach in hydroponics in a greenhouse. HortScience, 52(12): 1742-1747.

Gent, M.P.N. and Short, M.R. 2010. Managing a simple system to recycle nutrient solution to greenhouse tomato grown in rockwool. In XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010): International Symposium on, 927: 913-919.

Han, T., Vaganov, V., Cao, S., Li, Q., Ling, L., Cheng, X. and Tu, M. 2017. Improving color rendering" of LED lighting for the growth of lettuce. Scientific Reports, 7: 1, 1-7.

He, J., Qin, L. and Chow, W. S. 2019. Impacts of LED spectral quality on leafy vegetables: Productivity closely linked to photosynthetic performance or associated with leaf traits?. International Journal of Agricultural and Biological Engineering, 12(6): 16-25.

Hernández, R. and Kubota, C. 2016. Physiological responses of cucumber seedlings under different blue and red photon flux ratios using LEDs. Environmental and Experimental Botany, 121: 66–74.

Hernàndez, R., and Kubota, C. 2012. Tomato seedling growth and morphological responses to supplemental LED lighting red: Blue ratios under varied daily solar light integrals. Acta Horticulturae, 956: 187–194.

Hogewoning, S. W., G. Trouwborst, H. Maljaars, H. Poorter, W. van Ieperen, and J. Harbinson. 2010. Blue Light Dose-Responses of Leaf Photosynthesis, Morphology, and Chemical Composition of *Cucumis Sativus* Grown under Different Combinations of Red and Blue Light. Journal of Experimental Botany, 61: 3107-17.

Hussain, A., Iqbal, K., Aziem, S., Mahato, P., and Negi, A. K. A 2014. review on the science of growing crops without soil (soilless culture)-a novel alternative for growing crops. International Journal of Agriculture and Crop Sciences, 7: 11, 833.

Kang, W. H., Park, J. S., Park, K. S. and Son, J. E. 2016. Leaf photosynthetic rate, growth, and morphology of lettuce under different fractions of red, blue, and green light from light-emitting diodes (LEDs). Horticulture, Environment, and Biotechnology, 57(6): 573-579.

Kim, H.H., Goins, G.D., Wheeler, R.M. and Sager, J.C. 2004. Stomatal Conductance of Lettuce Grown under or Exposed to Different Light Qualities. Annals Botany, 94: 691-7.

Kim, M. J., Moon, Y., Tou, J. C., Mou, B. and Waterland, N. L. 2016. Nutritional value, bioactive compounds and health benefits of lettuce (*Lactuca sativa* L.). Journal of Food Composition and Analysis, 49: 19-34.

Łabędzki, Leszek, and Bogdan Bąk. 2017. Impact of Meteorological Drought on Crop Water Deficit and Crop Yield Reduction in Polish Agriculture. Journal of Water and Land Development, 34: 181.

Lamb, J.J., Røkke, G. and Hohmann-Marriott, M.F. 2018. Chlorophyll fluorescence emission spectroscopy of oxygenic organisms at 77 K. Photosynthetica, 56(1):105-124.

Lawson, T. and Blatt, M.R. 2014. Stomatal size, speed, and responsiveness impact on photosynthesis and water use efficiency. Plant physiology, 164(4): 1556-1570.

Lawson, T. and S. Vialet-Chabrand. 2019. Speedy Stomata, Photosynthesis and Plant Water Use Efficiency. New Phytologist, 22: 93-98.

Leong, T. Y., and Anderson, J. M. 1984. Effect of light quality on the composition and function of thylakoid membranes in *Atriplex triangularis*. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 766(3): 533-541.

Li, H., Tang, C., Xu, Z., Liu, X. and Han, X. 2012. Effects of different light sources on thegrowth of non-heading Chinese cabbage (*Brassica campestris* L.). The Journal of Agricultural Science, 4: 262–270.

Lightenthaler, H. K. 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods in Enzymology, 148: 350-382.

Matthews, J.S.A., Vialet-Chabrand, S. and Lawson, T. 2020. Role of Blue and Red Light in Stomatal Dynamic Behaviour. Journal of Experimental Botany, 71: 2253-69.

Mattson, N.S. and Peters, C.A.R.I. 2014. A recipe for hydroponic success. Inside Grower, 16-19.

Matysiak, B., Kaniszewski, S., Dyśko, J., Kowalczyk, W., Kowalski, A., and Grzegorzewska, M. 2021. The impact of LED light spectrum on the growth, morphological traits, and nutritional status of 'Elizium'romaine lettuce grown in an indoor controlled environment. Agriculture, 11(11): 1133.

Meng, X., Wang, Z., He, S., Shi, L., Song, Y., Lou, X., and He, D. 2019. LED-supplied red and blue light alters the growth, antioxidant status, and photochemical potential of in vitro-grown Gerbera jamesonii plantlets. Horticultural Science and Technology, 37: 473-89.

Miao, Y. X., Wang, X. Z., Gao, L. H., Chen, Q. Y. and Mei, Q. U. 2016. Blue light is more essential than red light for maintaining the activities of photosystem II and I and photosynthetic electron transport capacity in cucumber leaves. Journal of Integrative Agriculture, 15(1): 87-100.

Miras-Moreno, B., Corrado, G., Zhang, L., Senizza, B., Righetti, L., Bruni, R. and Lucini, L. 2020. The metabolic reprogramming induced by sub-optimal nutritional and light inputs in soilless cultivated green and red butterhead lettuce. International journal of molecular sciences, 21(17): 6381.

Moazzeni, M., Reezi, S. and Ghehsareh, M. G. 2020. Growth and chlorophyll fluorescence characteristics of Sinningia speciosa under red, blue and white light-emitting diodes and sunlight. Advances in Horticultural Science, 34(4): 419-430.

Mou, B. 2012. Nutritional Quality of Lettuce. Current nutrition and Food science, 8: 177-87.

Muneer, S., E. J. Kim, J. S. Park, and J. H. Lee. 2014. Influence of Green, Red and Blue Light Emitting Diodes on Multiprotein Complex Proteins and Photosynthetic Activity under Different Light Intensities in Lettuce Leaves (*Lactuca Sativa* L.). International Journal of Molecular Sciences, 15: 4657-70.

Naznin, M. T., Lefsrud, M., Gravel, V., and Azad, M.O.K. 2019. Blue light added with red LEDs enhance growth characteristics, pigments content, and antioxidant capacity in lettuce, spinach, kale, basil, and sweet pepper in a controlled environment. Plants, 8(4): 93.

Nguyen, T.P.D., Jang, D.C., Tran, T.T.H., Nguyen, Q.T., Kim, I.S., Hoang, T.L.H. and Vu, N.T. 2021. Influence of Green Light Added with Red and Blue LEDs on the Growth, Leaf Microstructure and Quality of Spinach (*Spinacia oleracea* L.). Agronomy, 11(9): 1724.

Oh, H. E., Yoon, A., and Park, Y. G. 2021. Red Light enhances the antioxidant properties and growth of *Rubus hongnoensis*. Plants, 10(12): 2589.

Pantanella, E, M Cardarelli, PP Danieli, A MacNiven, and G Colla. 2010. Integrated Aquaculture-Floating Agriculture: Is It a Valid Strategy to Raise Livelihood?" Paper presented at the XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010): International Symposium on, 921: 79-86.

Parolin, P, Waldhoff, D. and Maria, T.F. P. 2010. Gas Exchange and Photosynthesis. In Amazonian Floodplain Forests, 203-22.

Pennisi, G., Blasioli, S., Cellini, A., Maia, L., Crepaldi, A., Braschi, I., Spinelli, F., Nicola, S., Fernandez, J.A., Stanghellini, C. and Marcelis, L.F. 2019. Unraveling the role of red: blue LED lights on resource use efficiency and nutritional properties of indoor grown sweet basil. Frontiers in plant science, 10: 305.

Pérez-Urrestarazu, L., Lobillo-Eguíbar, J., Fernández-Cañero, R. and Fernández-Cabanás, V. M. 2019. Suitability and optimization of FAO's small-scale aquaponics systems for joint production of lettuce (Lactuca sativa) and fish (Carassius auratus). Aquacultural Engineering, 85: 129-137.

Piovene, C., Orsini, F., Bosi, S., Sanoubar, R., Bregola, V., Dinelli, G. and Gianquinto, G. 2015. Optimal red: blue ratio in led lighting for nutraceutical indoor horticulture. Scientia Horticulturae, 193: 202-208.

Porra, R.J., Thompson, W. A. and Kriedemann, P. E. 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 975(3): 384-394.

Prasad, A., Du, L., Zubair, M., Subedi, S., Ullah, A., and Roopesh, M. S. 2020. Applications of Light-Emitting Diodes (LEDs) in food processing and water treatment. Food Engineering Reviews, 12(3): 268-289.

Raschke, K. 1975. Stomatal Action. Annual Review of Plant Physiology, 26: 309-40.

Rehman, M., S. Fahad, M. Hamzah Saleem, M. Hafeez, MHu Rahman, F. Liu, and G. Deng. 2020. Red light optimized physiological traits and enhanced the growth of ramie (*Boehmeria nivea* L.). Photosynthetica, 58(4): 922-931.

Resh, H. M. 2022. Hydroponic food production: a definitive guidebook for the advanced home gardener and the commercial hydroponic grower. CRC press.

Ritchie, S. W., Nguyen, H. T., and Holaday, A. S. 1990. Leaf water content and gas-exchange parameters of two wheat genotypes differing in drought resistance. Crop science, 30(1): 105-111.

Roháček, K., Soukupová, J., and Barták, M. 2008. Chlorophyll fluorescence: a wonderful tool to study plant physiology and plant stress. Plant Cell Compartments-Selected Topics. Research Signpost, Kerala, India, 41-104.

Rolnik, A., and B. Olas. 2021. The Plants of the Asteraceae Family as Agents in the Protection of Human Health. International Journal of Molecular Sciences, 6: 3009.

Roni, M. Z. K., Islam, M. S., and Shimasaki, K. 2017. Response of Eustoma leaf phenotype and photosynthetic performance to LED light quality. Horticulturae, 3(4): 50.

Ronzoni, R. 2020. A Guide To Home Hydroponics For Leafy Greens. Master of Professional Studies In Agriculture and Life Science, Field of Horticulture, Cornell University, 101 pages.

Sabzalian, M., Heydarizadeh, P., Zahedi, M., Boroomand, A., Agharokh, M. and Sahba, M. Schoefs B. 2014. High performance of vegetables, flowers, and medicinal plants in a red-blue LED incubator for indoor plant production. Agronomy Sustainable Development, 34(14): 879–886.

Shimazaki, K., Doi, M., Assmann, S.M. and Kinoshita, T. 2007. Light Regulation of Stomatal Movement. Annual Review of Plant Biology, 58: 219-47.

Silva, L., Gasca-Leyva, E., Escalante, E., Fitzsimmons, K. M., and Lozano, D. V. 2015. Evaluation of biomass yield and water treatment in two aquaponic systems using the dynamic root floating technique (DRF). Sustainability, 7(11): 15384-15399.

Son, K. H., and Oh, M. M. 2015. Growth, photosynthetic and antioxidant parameters of two lettuce cultivars as affected by red, green, and blue light-emitting diodes. Horticulture, Environment, and Biotechnology, 56(5): 639-653.

Stangl, Z.R., Tarvainen, L., Wallin, G., Ubierna, N., Räntfors, M. and Marshall, J.D. 2019. Diurnal variation in mesophyll conductance and its influence on modelled water-use efficiency in a mature boreal Pinus sylvestris stand. Photosynthesis Research, 141: 53-63.

Strasser, Reto J, Merope Tsimilli-Michael, and Alaka Srivastava. 2004. Analysis of the Chlorophyll a Fluorescence Transient. In Chlorophyll a Fluorescence, 321: 62.

Trejo-Téllez, L.I. and Gómez-Merino, F.C. 2012. Nutrient solutions for hydroponic systems. Hydroponics-a Standard Methodology for Plant Biological Researches, 1-22.

Tsai, Y.C., Chen, K.C., Cheng, T.S., Lee, C., Lin, S.H. and Tung, C.W. 2019. Chlorophyll fluorescence analysis in diverse rice varieties reveals the positive correlation between the seedlings salt tolerance and photosynthetic efficiency. BMC Plant Biology, 19: 1-17.

Van der Lugt, G., Holwerda, H.T., Hora, K., Bugter, M., Hardeman J. and Vries, P.De. 2020. Nutrient Solutions for Greenhouse Crops. Version 4. Pp 1-98. ISBN 9789464021844 Made available by: Eurofins Agro, Geerten van der Lugt, Nouryon, SQM, Yara.

Van der Tol, C., Verhoef, W. and Rosema, A. 2009. A Model for Chlorophyll Fluorescence and Photosynthesis at Leaf Scale. Agricultural and Forest Meteorology, 149: 96-105.

Vernay, A., Tian, X., Chi, J., Linder, S., Mäkelä, A., Oren, R., Peichl, M., Stangl, Z.R., Tor-Ngern, P. and Marshall, J.D. 2020. Estimating canopy gross primary production by combining phloem stable isotopes with canopy and mesophyll conductances. Plant, Cell and Environment, 43(9): 2124-2142.

Wang, H., Gu, M., Cui, J., Shi, K., Zhou, Y. and Yu, J. 2009. Effects of light quality on CO_2 assimilation, chlorophyll-fluorescence quenching, expression of Calvin cycle genes and carbohydrate accumulation in *Cucumis sativus*. Journal of Photochemistry and Photobiology B: Biology, 96(1): 30-37.

Wang, J., Lu, W., Tong, Y.X., and Yang, Q.C. 2016. Leaf morphology, photosynthetic performance, chlorophyll fluorescence, stomatal development of lettuce (*Lactuca sativa* L.) exposed to different ratios of red light to blue light. Front. Plant Science, 7: 250.

Wang, J., Lu, W., Tong. Y. and Q. Yang. 2016. Leaf Morphology, Photosynthetic Performance, Chlorophyll Fluorescence, Stomatal Development of Lettuce (*Lactuca Sativa* L.) Exposed to Different Ratios of Red Light to Blue Light. Frontiers in Plant Science, 7: 250.

Wang, M., H. Wei, and B. R. Jeong. 2021. Lighting Direction Affects Leaf Morphology, Stomatal Characteristics, and Physiology of Head Lettuce (*Lactuca Sativa* L.). International Journal of Molecular Sciences, 22: 3157.

Wang, X.Y., Xu, X.M. and Cui, J. 2015. The importance of blue light for leaf area expansion, development of photosynthetic apparatus, and chloroplast ultrastructure of Cucumis sativus grown under weak light. Photosynthetica, 53: 213–222.

Ward, J.M., Cufr, C.M., Denzel, M.A. and Neff, M.M. 2005. The dof transcription factor OBP3 modulates Phytochrome and cryptochrome signaling in arabidopsis. Plant Cell, 17: 475–485.

Williams, M., Waters, E., Golbek, M. and Wormington, J. 2015. Effect of different shades of light on photosynthesis. Journal of Introductory Biology Investigations, 2(4): 1-15.

Wojciechowska, R., Kołton, A., Długosz-Grochowska, O., Kunicki, E., Mrowiec, K. and Bathelt, P. 2020. LED lighting affected the growth and metabolism of eggplant and tomato transplants in a greenhouse. Horticultural Science, 47: 3.

Wojciechowska, R., Kolton, A., Dlugosz-Grochowska, O., Zupnik, M. and Grzesiak, W. 2013. The effect of LED lighting on photosynthetic parameters and weight of lamb's lettuce (*Valerianella locusta*). Folia Horticulturae, 25, 1, 41.

Yang, X., M. I. Gil, Q. Yang, and F. A. 2022. Tomás-Barberán. "Bioactive Compounds in Lettuce: Highlighting the Benefits to Human Health and Impacts of Preharvest and Postharvest Practices. Comprehensive Reviews in Food Science and Food Safety, 21: 4-45.

Yu, H. and Ong, B.L., 2003. Effect of radiation quality on growth and photosynthesis of Acacia mangium seedlings. Photosynthetica, 41: 349-355.

Zarco-Tejada, P. J., Catalina, A., González, M. R. and Martín, P. 2013. Relationships between net photosynthesis and steady-state chlorophyll fluorescence retrieved from airborne hyperspectral imagery. Remote Sensing of Environment, 136: 247-258.

Zhang, S., Ma, J., Zou, H., Zhang, L., Li, S., and Wang, Y. 2020. The combination of blue and red LED light improves growth and phenolic acid contents in Salvia miltiorrhiza Bunge. Industrial Crops and Products, 158: 112959.

Zhang, Z., Zhang, L., Xu, H., Creed, I.F., Blanco, J.A., Wei, X., Sun, G., Asbjornsen, H. and Bishop, K. 2023. Forest water-use efficiency: Effects of climate change and management on the coupling of carbon and water processes. Forest Ecology and Management, 534: 120853.

Zheng, L., & M. C. Van Labeke. 2017. Long-Term Effects of Red and Blue Light Emitting Diodes on Leaf Anatomy and Photosynthetic Efficiency of Three Ornamental Pot Plants. Front in Plant Science, 8: 917.

Zheng, Y., M. Xu, R. Hou, R. Shen, S. Qiu, and Z. Ouyang. 2013. Effects of Experimental Warming on Stomatal Traits in Leaves of Maize (*Zea May* L.). Ecology and Evolution, 3095-111.

Zhushi Etemi, F., Çadraku, H., Bytyçi, A., Kuçi, T., Desku, A., Ymeri, P., and Bytyçi, P. 2020. Correlation between physical and chemical parameters of water and biotic indices: The case study the White Drin River basin, Kosovo. Journal of Water and Land Development, 7: 229-241.