

Growth and photosynthesis characteristics of lettuce cultivars in response to supplementary LED lights and replacement methods of the nutrient solution

Hamid Reza Soufi^{a*}, Awais Ali^b, Mahdiyeh Shojaee^a

^a Department of Horticultural Sciences, Faculty of Agriculture, Vali-e-Asr University of Rafsanjan, Rafsanjan, Iran.

^b Department of Agricultural and Environmental Sciences, Università degli Studi di Milano, Via Celoria 2, 20133 Milano, MI, Italy

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ABSTRACT

Growing global warming and a projected rise in the world's population have made it imperative to produce food and agricultural products with a limited supply of water and energy sources. Conversely, there is a need to employ modern technologies, such as LED artificial lights, to ensure that plants receive adequate light energy and do not experience low light stress during the growing season, especially winter. Therefore, this research was aimed to investigate the effects of supplementary LED light application (white, a combination of red and blue, monochromatic red and monochromatic blue) and three replacement methods of nutrient solution of aeroponic system (complete replacement of the nutrient solution, based on electrical conductivity (EC) and based on plant needs) on three lettuce cultivars (Red Sail, Edox and Summer Bibb). The results showed that white light significantly affected the fresh shoot and root weight in Red Sails variety under the complete replacement method of the nutrient solution. The highest photosynthetic pigments concentrations were observed under the combination of red and blue and for monochromatic red light. Photosynthetic gas exchange was improved under white light and in all three replacement methods of nutrient solutions whereas the most positive effect was recorded in the replacement method based on plant needs. Therefore, the simultaneous use of supplemental lights and replacement methods of nutrient solution, particularly replacement based on the plant needs improves lettuce growth and quality.

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* Corresponding author: H.R. Soufi

E-mail address: hamidsoufi70@gmail.com

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1. Introduction

In addition to providing plants with a basic energy source for photosynthesis, light plays a crucial role in regulating plant functions and guiding different growth processes (Li *et al.*, 2012). The required light for plant growth is however, provided either naturally by the sun or through the use of artificial lights (Kim *et al.*, 2004). Seasonality of light is a common challenge for greenhouse production, especially in high latitudes and areas with cloudy periods. On a cloudy day, an insufficient light intensity and a short duration of lighting may impose limitations on photosynthesis (Esmaili *et al.*, 2022; Javadi Asayesh *et al.*, 2021). Researchers found that using supplemental light can improve the growth and performance characteristics of greenhouse sweet pepper cultivars in winter (Adibian *et al.*, 2023). In low light conditions that generally occurred during the winter months, the use of artificial light is crucial to promote plant productivity (Chia and Kubota, 2010). Scientifics believed that decreasing light intensity in winter and in the growth environment caused a reduction in the biomass in *Salvia officinalis* L. (Zervoudakis *et al.*, 2012). To boost photosynthesis and guarantee economical yield in areas where the natural environmental conditions do not provide enough sunlight, application of artificial light through supplemental lighting is a common practice (Guiamba *et al.*, 2022). Hence, the use of supplementary lighting is important among the automation systems used in greenhouses (Koksal *et al.*, 2015). Recently, LED lamps have been widely installed in greenhouses in Iran due to their efficiency and environment friendly nature than other existing artificial lighting sources such as HPS, TFL, MH etc. LEDs have been used for plant growth and are known to have better results in terms of energy consumption compared to fluorescent lamps (Massa *et al.*, 2008; Olvera-Gonzalez *et al.*, 2013; Ali *et al.* 2023a). Furthermore, they are well known for saving up to 75% and 30% energy, respectively. In addition, the life of a standard lamp is about 1000 hours, while the average life of LED lamps is 20,000-50,000 hours (Köksal *et al.*, 2013). LEDs have several other advantages such as small size, specific wavelength, low heat output, adjustable light intensity and quality, as well as high photoelectric conversion efficiency (Yeh and Chung, 2009). Additionally, LED lamps provide lighting without harming plants as they do not emit UV or infrared radiation and are free from mercury or lead (Yeh and Chung, 2009). Also azizi et el (2024) reported that By utilizing lighting technology, growers can gain various positive outcomes, such as strategic production (with options for early flowering, continuous production, and consistent yields), enhanced plant structure (improved root growth and size, stem elongation, etc), determination of leaf and flower color, and elevated product quality. Likewise, LED energy sources can be supplied with solar panels, which allow these systems to be placed in tunnels outside the field and away from the energy grid. Researchers believed that supplemental LED lighting significantly increased plant biomass weight, flower number and leaves number in pansy plant (Koksal *et al.*, 2015), lettuce (Abdur Razzak *et al.*, 2022; Ali *et al.*, 2023b). Zheng *et al* (2015) indicated that white LEDs proved more efficient for romaine lettuce growth than red or blue LEDs. In another study, researchers believed that plants grown under W and B LEDs had a significantly higher number of leaves, plant weight, and chlorophyll content than plants grown in a greenhouse (Luković *et al.*, 2024). Scientific research indicated that LEDs are the great source of light for improving lettuce productivity and quality improvement (Chen *et al.*, 2023; Hernández-Adasme *et al.*, 2023; Jin *et al.*, 2023; Miao *et al.*, 2023). Now a days, greenhouses all around the world use hydroponics, a technique that doesn't require soil as a substrate but utilizes water as a growing medium. Its examples include Nutrient film technique (NFT), ebb-and-flow system, deep water culture (DWC), drip system, wicking system, aeroponic, and aquaponics (El-Kazzaz & El-Kazzaz, 2017).

Lettuce (*Lactuca sativa* L.) is one of the most cultivated greenhouse crops worldwide (Naznin *et al.*, 2019). The production of lettuce in greenhouses is already undergoing a highly

automated process. Multiple growing systems can be used for lettuce production in greenhouses, including hydroponic, aquaponic, and vertical growing systems (Petrovoulou *et al.*, 2023). This automation has led to a significant decrease in labor costs and has made higher-quality production possible. Additionally, it has made it possible to grow lettuce year-round, regardless of the weather outside. Production of lettuce in greenhouses is limited when the light requirements of this crop are not considered due to seasonal light limitations or greenhouse structural restrictions (Sheikhi *et al.*, 2024).

Aeroponics is the practice of growing plants in an air or mist environment without the use of any substrate in which the plant roots are suspended in the air and are misted or sprayed periodically with a nutrient solution or aerosol of nutrient solution (Ali *et al.* 2024). When compared to soil-cultivated plants, these cultivation systems provide improved growth, yield, quality, and metabolite production (Partap *et al.*, 2020; Thakur *et al.*, 2019). In aeroponics system, the plants have 100% access to CO₂ (450 ppm to 780 ppm) for photosynthesis and consume 70% less water than hydroponics. In this method, not only is the consumption of water and nutrients reduced, but conversely, the transmission of fungi and bacteria is significantly reduced. Now a days, many methods have been investigated to reduce the consumption of water and nutrients without compromising the growth, development and production of agricultural products. One of these replacing methods is the nutrient solution, which can significantly reduce the consumption of water and nutrients. Managing nutrients in recirculating solutions can be challenging in hydroponic production, and poor management practices can rapidly result in root zone nutrient imbalances that can result in reduced yield. Using mass balance principles to formulate hydroponic replenishment solutions is a proposed strategy to reduce nutrient imbalances and the need to periodically replace the hydroponic solution (Houston *et al.*, 2023). On the other hand, automatically replacing the used water prevents the accumulation of elements, but it requires compensation for the useful drained elements. It has been recommended that determination of each element concentration should be started during the use of nutrient solution, and according to the amount of absorption of the elements, a filler solution that contains all or some of the elements depleted from the nutrient solution should be added to the initial starter solution (Bugbee, 2004). Savvas (2002) proposed two models for preparation of the additive solution, which was based on the precise monitoring of individual elements in the drained nutrient solution. Moreover, although element monitoring can be very helpful, Bugbee (2004) stated that it is not essentially required, instead it was recommended using an additive solution with concentrations lower than the original solution concentration to make up for the decrease in nutrient solution volume.

Application of circulation system and replacement of the nutrient solution every four weeks caused an increase in EC and the accumulation of some ions and showed a negative effect on the yield and quality of tomato fruits (Lopez *et al.*, 2003). For arugula (*Eruca sativa* L.) and basil (*Ocimum basilicum* L.), species-specific replenishment solutions led to a gradual decline in the EC of the solution and concentrations of most macronutrients, while replenishment treatment had no effect on yield (Houston *et al.*, 2023). Therefore, considering the high waste of nutrient solutions, the high waste of water and nutrient elements, the pollution of the surrounding environment, as well as the high consumption of electricity and the production of high temperatures in greenhouses with high-pressure sodium lamps, this research aims to increase the growth and photosynthetic characteristics of lettuce cultivars under the influence of different supplementary LED lights and different methods of nutrient and solution replacement in aeroponic conditions in the greenhouse.

2. Materials and Methods

2.1. Plant materials, growth conditions, and experimental design

This experiment was conducted in a greenhouse of Vali-e-Asr University in winter of 2020. The seeds of lettuce varieties (Red Sail (Leaf lettuce) and Edox and Summer Bibb (Butterhead Lettuce)) were purchased from Sepahan Rooyesh Co, Isfahan (Iran). The seeds were sown into the seed tray filled with fine perlite medium. Normally, after the four-leaf stage, the seedlings were transplanted inside the small plastic net pot containing horticultural grade perlite medium. These small pots were situated into holes in the aeroponic system. Three plastic tubs (dimensions: 1500 × 500 × 500 cm, respectively, length, width, and height) were used to produce and grow lettuce varieties in the aeroponic system. Inside each plastic tub, four nozzles with 30 cm intervals were installed to direct water and nutrient solutions in the form of powder towards the roots. To keep the plants in the aeration system, a Styrofoam plate was used with 5x5 holes. At the bottom of each tank, there was a 100-liter tank, which was used based on the replacement treatments of different nutrient solutions, and the solution using a pump, it was directed to the nozzles and using a special timer, the nutrient solution was sprayed to the root every 15 minutes for 15 seconds. This nutrient solution returned to the reservoir tank through gravity flow. After transferring the plants to the aeroponic systems, the Resh nutrient solution (EC: 2.1 ds m⁻¹, pH: 7) formulated for lettuce was used (Resh, 2022). EC and pH were monitored daily during the cultivation process with portable pH meter (Fisherbrand™ accumet™ AP115 Portable pH Meter Kit) and portable EC meter (HI9033 EC Meter, Setare Arsh Aria company). The nutrient solution (5 mM KNO₃, 5 mM Ca (NO₃)₂, 2 mM MgSO₄, 1 mM KH₂PO₄, 7 μM MnCl₂, 0.7 μM ZnSO₄, 0.8 μM CuSO₄, 0.8 μM Na₂MoO₄, 25 μM Fe-EDDHA, and 2 μM H₃BO₃) was applied to the seedlings immediately after transplanting. Deionized water was used for nutrient preparation. After transplanting (seedling in four leaves stage) of lettuce varieties, nutrient solutions were replaced by three different replacement methods (complete replacement, partial replacement based on EC, and partial replacement according to plant needs) for 40 days. In complete nutrient solution replacement treatment, the nutrient solution was replaced weekly. For partial replacement according to EC, the EC of the nutrient solution was adjusted to 2.2 dS m⁻¹ by adding predetermined amounts of potassium sulfate, calcium nitrate, magnesium sulfate, potassium dihydrogen phosphate, and half strength of micronutrient solution every 48 h. In the replacement treatment based on the needs of the plants, potassium nitrate is used at the same concentration as in the original solution, and the amount of calcium nitrate, magnesium sulfate, and potassium dihydrogen phosphate was reduced by three quarters and the microelements (Fe, Zn, Cu, and Mn) were reduced by half and added every 2 days based on the amount of water added to the plastic container in which the plants were cultivated. Environmental conditions in the greenhouse were a temperature of 25/15 (day/night), a photoperiod of 16/8 h (day/night), and a relative humidity of 50 ± 5%.

2.2. LED tubes and light treatments

Lettuce plants were cultivated under supplementary LED lamps with a power of 24 W (Parto Roshd Novin Ltd Co., Iran) at different spectral ranges [red (R, peak at 656 nm), blue (B, peak at 450 nm), red/blue (3:1) (R: B), and white (400–700 nm)] in the winter of 2020. Ambient light was considered as the control. The LED lamps were installed at a distance of 30 cm from the plant canopy to make the optimum and same photosynthetically active radiation (PAR) which was 215 ± 5.5 μmol m⁻² s⁻¹ for all applied treatments. Details on the

characteristics of LEDs used in the present study are given in Table 1 and Figure 1.

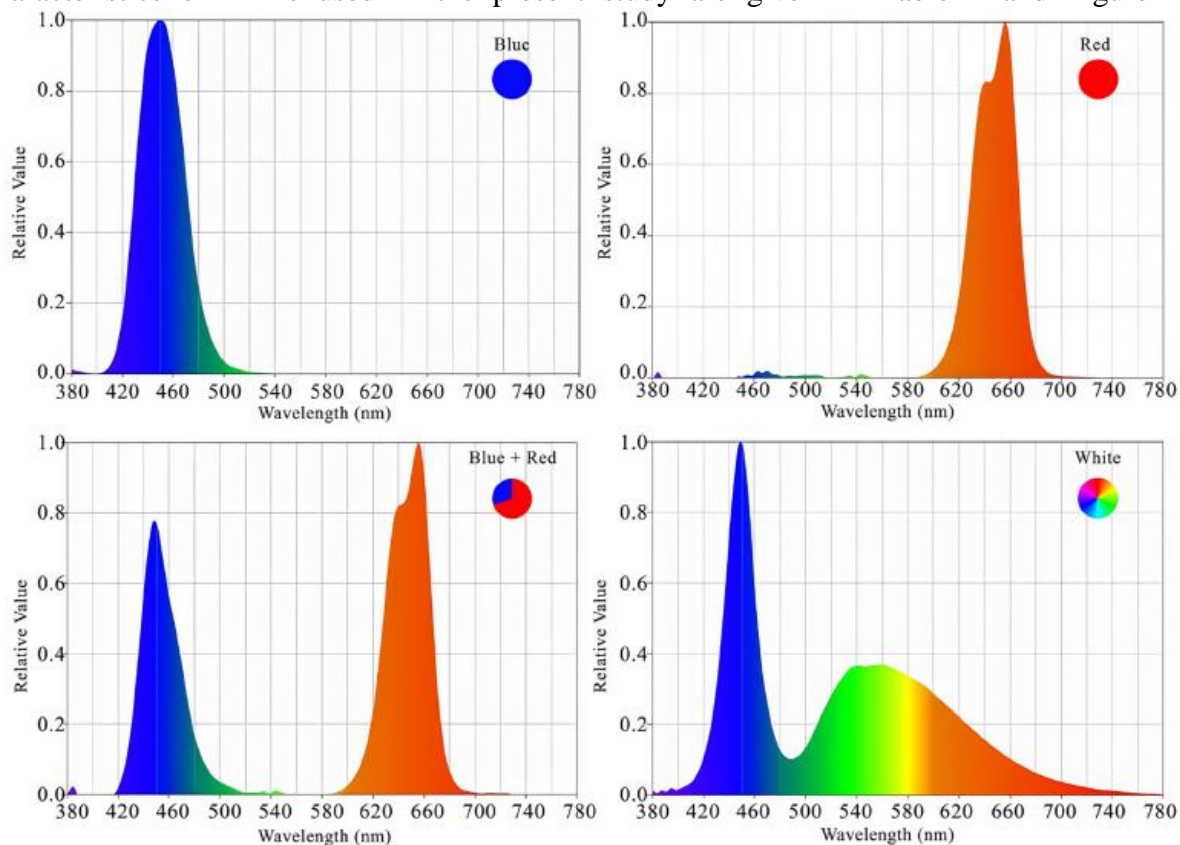


Figure 1. Relative distribution of different LED spectra (red, red/blue 3:1, blue and white) used during the experiment.

Table 1. Characteristics of LEDs used in this experiment.

Manufacture company	Efficiency	N. of LEDs	Light coverage area	Power consumption	Lens type	DC Voltage
Iran Grow Light	90%	24	40×10 cm	24 W	90°	54–84V

2.3. Vegetative characteristics

At the end of the experiment (40 days after transplanting), the plants were harvested from each aeroponic system and divided into shoots and roots.

2.4. 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 (Sigma Co., Germany) at 350 rpm for 10 minutes and then the samples were 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.

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

$$\text{Chlorophyll a (Chl a)} = [(12.25 \times \text{OD}_{663.6}) - (2/22 \times \text{OD}_{646.6})] \times [V/W]$$

$$\text{Chlorophyll b (Chl b)} = [(\text{OD}_{646.6} \times 20.31) - (\text{OD}_{663.9} \times 91.4)] \times [V/W]$$

$$\text{Carotenoid (Car)} = (1000 \text{ OD}_{470} - 3.27 [\text{chl a}] - 104 [\text{chl b}])/227$$

OD: read absorbance V: the volume of acetone used W: sample mass (grams)

2.5. Leaf gas exchange parameters

Photosynthetic and gas exchange parameters were determined using an Infra-red Gas Analyzer System (IRGAs, LCI Ultra Compact, ADC BioScientific Limited, Hertfordshire, UK) (with use of PAR control by LED light: up to $2400 \mu\text{mol m}^{-2} \text{s}^{-1}$ RGB LED array, or up to $2500 \text{ m}^{-2} \text{s}^{-1}$ by white LED array; CO_2 concentration: 400 ppm; chamber temperature: 20 ± 3 and precision thermistor (0.2°C accuracy; H_2O : 30 mbar and 0.1 mbar resolution; two laser-trimmed, fast response water vapor sensors: Direct leaf temperature: 25°C self-positioning microchip thermistor/energy balance/manually positioned thermistor; flow rate to leaf chamber: 68 to $340 \mu\text{mol m}^{-2} \text{s}^{-1}$; and gas connections: 3 mm barbed) following the procedure used by Afridi *et al.* (2020). Around 9:00 a.m. and 12:00 a.m., measurements were performed on completely expanded leaves. Briefly, one fully expanded and intact leaf was selected from each replicate and clamped to the leaf chamber of the instrument. After 5 min (for warm up at 20°C) and 15 min adaptation to achieve stable steady-state conditions, net photosynthetic rate (P_N ; $\mu\text{mol m}^{-2} \text{mol}^{-1}$), stomatal conductance (g_s ; $\text{mol m}^{-2} \text{s}^{-1}$), transpiration rate (E ; $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$), intercellular CO_2 concentration (C_i ; $\mu\text{mol CO}_2 \text{mol}^{-1}$) were noted.

2.6. Statistical analysis

This study was arranged in a three factorial experiment based on completely randomized design (CRD) with three replications ($n = 3$). SAS software version 9.4 was used to analyze the obtained data (SA institute, Cary, NC, USA). A two-way ANOVA model was used to test the statistical analysis of data. Duncan's multiple-range test (DMRT) was used as a post hoc test to determine the specific differences between group means. Differences were considered significant at $p \leq 0.05$. Multivariate analyses of variance were performed using XLSTAT software (Addinsoft, New York, USA). The results were expressed as mean values and standard errors (SE) of the means.

3. Results

3.1. Fresh shoot and root

The fresh weight of shoots and roots was influenced by the experimental factors and the interaction between them (Table 2). Thus, the highest fresh weights of shoots and roots were observed under the influence of white light and in Red Sails variety under the treatment of complete replacement method of nutrient solution (Table 4). Also, in the condition of using monochromic red light, the use of replacement method of nutrient solution based on EC and in Red Sails and Edox varieties caused an increase in shoot fresh weight, but in these spectra of light, the fresh weight of roots in replacement method based on the plant needs and in Summer Bibb showed an increase (Table 4). Also, the results showed that in combination of red and blue light and monochromatic blue light conditions, in the complete replacement of the nutrient solution, the highest weight of shoots and roots was determined in the Red Sails variety (Table 4).

Table 2. Effect of supplemental light and replacement methods of nutrient solution on the growth and photosynthesis characteristics of lettuce varieties in aeroponic system.

Source of variation	DF	Fresh shoot	Fresh root	Chl <i>a</i>	Chl <i>b</i>	Total Chl	Car
Light (L)	3	**	**	**	**	**	**
Nutrient solution (N)	2	**	**	**	**	**	**
Varieties (V)	2	**	**	**	**	**	**
L*N	6	**	**	**	**	**	**
L*V	6	**	**	**	**	**	*
N*V	4	**	**	**	**	*	**
L*N*V	12	**	**	**	**	*	**
Experimental Error	72	3.87	0.60	100.2	90.07	76.09	33.83
Coefficient Variance (%)	-	8.75	14.46	13.44	15.8	20.92	28.59

*and** are significant at the five percent and one percent probability levels, respectively.

Table 3. Effect of supplemental light and replacement methods of nutrient solution on the Photosynthetic properties of lettuce varieties in aeroponic system. *PN* (net photosynthetic rate), *gs* (stomatal conductance), *E* (transpiration rate), *Ci* (intercellular CO₂ concentration).

Source of variation	df	SPAD	<i>PN</i>	<i>E</i>	<i>gs</i>	<i>Ci</i>
Light (L)	3	**	**	**	**	**
Nutrient solution (N)	2	**	**	**	**	ns
Varieties (V)	2	**	**	**	**	ns
L*N	6	**	**	**	**	**
L*V	6	**	**	**	**	ns
N*V	4	**	**	**	**	ns
L*N*V	12	**	**	**	**	ns
Experimental Error	72	1.16	0.44	0.29	0.016	32.93
Coefficient Variance (%)	-	6.5	7.61	8.39	8.96	11.61

*and** are significant at the five and one percent probability levels, respectively and ^{ns} not significant.

3.2. Photosynthetic pigments

The results of the variance analysis table (Table 2) showed that supplemental light, replacement of nutrient solution, lettuce variety and the interaction between these factors had a significant effect on photosynthetic pigments. Also, the results showed an increase in the content of total chlorophyll and chlorophyll *a* under the treatment of the Summer Bibb variety with red monochromatic light and the combination of red and blue light in replacement method based on the plant needs (Table 4). On the other hand, an increase in the content of total chlorophyll and chlorophyll *a* was observed in Red Sails cultivar under the treatment of red and blue light combination in all three replacement methods of nutrient solution (Table 4). Under the treatment using white light, the results showed that the use of replacement method based on EC caused an increase in the concentration of total chlorophyll and chlorophyll *a* in Summer Bibb variety (Table 4). In addition, it was found that blue light showed the lowest amounts of total chlorophyll and chlorophyll *a* in all three replacement methods of nutrient solution and in all three varieties of lettuce (Table 4). It was also noted that the use of a combination of red and blue light and monochromatic red light caused an increase in chlorophyll *b* in the Summer Bibb variety under replacement method based on the plant needs (Table 4). In addition, it was found that the use of white light in all three replacement methods of nutrient solution caused an increase in the content of chlorophyll *b* in the Red Sails variety (Table 4). Also, in the monochromatic red-light treatment, the use of replacement method based on the plant needs caused an increase in the concentration of chlorophyll *b* (Table 4). The use of red-light combination in the condition of complete replacement of nutrient solution and replacement based on the plant needs in the variety Red Silas caused an increase in the

concentration of chlorophyll *b*. Based on the results, it was found that the lowest concentration of chlorophyll *b* was observed in all three lettuce varieties under the influence of all three replacement methods of nutrient solution (Table 4). The carotenoid concentration showed a significant increase under the treatment of the combination of blue and red light and monochromatic red light in the condition of replacing method-based plant needs and in the Summer Bibb variety (Table 4).

In addition, in the conditions of using white light and in all three replacing methods the nutrient solution, the variety Red Sills showed high carotenoids content (Table 4). Under the condition of using monochromatic red light, in the treatment of complete replacement, replacement based on EC and replacement method based on nutritional needs, Summer Bibb, Red Sails and Summer Bibb yielded the highest content of carotenoid. But in the treatment of using combined blue and red light in the cultivars Summer Bibb, Red Sails and Edox, the use of replacement based on plant needs, complete replacement of nutrient solution, and replacement based on EC caused an increase in carotenoid content, respectively (Table 4). Blue light, however, caused a significant decrease in carotenoid content in all three replacement methods of nutrient solution and in all three lettuce varieties (Table 4).

3.3. SPAD (Greenness index)

Greenness index (SPAD) in this research was influenced by experimental factors (Table 3). Also, it was found that the use of white light and the combination of red and blue light in all three replacing methods of the nutrient solution in the Summer Bibb variety caused an increase in the greenness index (SPAD) (Table 5). In addition, in the condition of using monochromatic red light, the use of complete replacement of nutrient solutions in Red Sails variety, EC-based replacement in Red Sails variety and replacement of nutrient solutions based on the plant needs in Summer Bibb variety caused an increase in SPAD. Also, the lowest amounts of SPAD were observed under blue light treatment and in all three replacement methods of nutrient solution and in all three lettuce varieties (Table 5).

3.4. Leaf gas exchange parameters

The results of the present study showed that the P_N , E and g_s were affected by the experimental factors (Table 3). Also, the results of the average data comparison showed that the P_N in all three methods of replacement of nutrient solution and in white light and in all three varieties of lettuce, the P_N was higher than other supplemental lights (Table 5). In addition, it should be noted that in the Summer Bibb cultivar and in all three replacement methods of nutrient solution and under white light treatment was higher than other treatments (Table 5). The use of red monochromatic light on the redox variety in the condition of complete replacement of the nutrient solution, Summer Bibb in the condition of replacement method based on EC and in the condition of replacement based on the plant needs also improved the net photosynthesis rate (Table 5). On the other hand, irradiating a combination of red and blue light on the redox variety in the condition of complete replacement of the nutrient solution, Red Sails in the condition of replacement method based on EC and Summer Bibb in the condition of replacement method based on plant needs had a higher net photosynthetic rate than other treatments (Table 5). Also, the lowest P_N was observed in the monochromatic blue light treatment, but in this light spectrum, the Summer Bibb cultivar had a higher net photosynthesis rate than other treatments in all three replacement methods of the nutrient solution (Table 5).

Table 4. Changes in growth and photosynthesis pigments of lettuce cultivars in aeroponic system under the influence of supplemental light and replacement methods of nutrient solution. Values are means of three replicates. Different letters in each column show significant differences at $P \leq 0.05$ (LSD).

Supplementary Light	Replacement Methods of nutrient solution	Lettuce Cultivars	Fresh shoot (g)	Fresh root (g)	Total Chl (mg/gFW)	Chl <i>a</i> (mg/gFW)	Chl <i>b</i> (mg/gFW)
White	Complete replacement	Red Sails	124.7a	11.6a	958e-h	726def	229c-i
		Edox	92.2d	6.9ef	384mn	297k	86i-m
		Summer Bibb	5.2st	0.6p-r	888f-j	677d-h	209c-k
	Based on EC	Red Sails	57.2fgh	5.3h-k	709j-l	597.2f-j	110h-m
		Edox	80.15e	7.9d	373mn	489ij	102h-m
		Summer Bibb	15.4p-r	0.07p-r	1023d-g	804cde	217c-j
	Based on plant needs	Red Sails	110.5b	10.2v	731jk	456j297k	273c-f
		Edox	43.03ij	4.8jk	717jk	565f-j	150e-m
		Summer Bibb	42.8ij	1.7op	301no	239k-m	61klm
Red	Complete replacement	Red Sails	41.7ijk	5.5g-j	708j-l	529hij	177d-l
		Edox	38.6ijk	2.8n	638kl	269kl	147f-m
		Summer Bibb	5.2ts	0.4r	1034 d-g	800cde	234c-i
	Based on EC	Red Sails	59.7fg	5.2h-k	927f-i	714d-g	211c-j
		Edox	52.9h	4.5jk	811g-k	646e-i	164d-m
		Summer Bibb	11.4qrs	1.02p-r	767i-k	553g-j	212c-j
	Based on plant needs	Red Sails	17.7op	1.0p-r	1050def	805cde	242c-h
		Edox	45.7i	4.2kl	1117de	847bcd	267c-f
		Summer Bibb	38.1jk	6.0f-i	1666a	1119a	543a
70 Red/ 30 Blue	Complete replacement	Red Sails	100.2c	9.02c	1397b	1085a	308bcd
		Edox	53.5gh	6.7ef	534lm	524hij	311m
		Summer Bibb	2.8t	0.26r	886f-j	772cde	112g-m
	Based on EC	Red Sails	61.02f	6.2e-h	1171cd	907bc	261c-g
		Edox	35.13kl	4.2kl	1324bc	991ab	330bc
		Summer Bibb	12.5pqr	1.0p-r	857g-j	678d-h	177d-l
	Based on plant needs	Red Sails	23.7o	1.5o-q	1482b	1064a	414b
		Edox	56.2fgh	5.0i-k	976e-h	676d-h	298b-e
		Summer Bibb	73.9e	7.3de	1349b	1097a	634a
Blue	Complete replacement	Red Sails	58.2fgh	6.5efg	319n	242k-m	76j-m
		Edox	30.7lm	2.3no	128op	99lm	28lm
		Summer Bibb	3.18t	0.23r	296no	225k-m	69j-m
	Based on EC	Red Sails	19.07o	1.7n-p	236n-p	199k-m	36lm
		Edox	26.7mn	2.6m-o	124op	89m	34lm
		Summer Bibb	5.15ts	0.24r	341n	268kl	72j-m
	Based on plant needs	Red Sails	43.4ij	3.4l	243n-p	152k-m	91i-m
		Edox	14.3pqr	1.6o-q	239n-p	188k-m	50lm
		Summer Bibb	14.2pqr	0.5qr	100p	79m	20m

Table 5. Changes in Photosynthetic pigments and plant gas exchange parameters of lettuce cultivars in aeroponic system under the influence of supplemental light and replacement methods of nutrient solution. Values are means of three replicates. Different letters in each column show significant differences at $P \leq 0.05$ (LSD). Car (Carotenoid).

Supplementary Light	Replacement Methods of nutrient solution	Lettuce Cultivars	Car (mg/gFW)	Spad	P_N ($\mu\text{mol m}^{-2} \text{mol}^{-1}$)	E (mol $\text{H}_2\text{O m}^{-2} \text{s}^{-1}$)	gs (mol $\text{m}^{-2} \text{s}^{-1}$)
White	Complete replacement	Red Sails	107.9c-i	25.8e	5.8f	3.0jk	0.21fg
		Edox	41.8i-o	16k	8.7cd	4.1fg	0.33cd
		Summer Bibb	98.5c-k	30.3bc	11.5a	4.4ef	0.32cde
	Based on EC	Red Sails	53.1h-o	20.46h	11.1a	4.5ef	0.31de
		Edox	47.7h-o	12lmn	10.3b	5.4bc	0.4b
		Summer Bibb	10.7c-i	35.2a	11.1a	5.3bc	0.34c
	Based on plant needs	Red Sails	125.1c-e	16.2k	9.1c	5.8b	0.41ab
		Edox	72.0e-o	10mno	9.2c	5.3bc	0.30e
		Summer Bibb	29.1l-o	28.9cd	11.8a	6.4a	0.43a
Red	Complete replacement	Red Sails	83.5d-m	23.9f	5.5fg	1.9lmn	0.111-p
		Edox	68.9f-o	12.9l	8.4cd	4.7e	0.21f
		Summer Bibb	111.5c-h	21.3gh	5.0gh	4.77de	0.22f
	Based on EC	Red Sails	100.4c-j	30bcd	4.0ij	5.3c	0.18gh
		Edox	78.3e-n	12.8l	2.4mn	5.2cd	0.16hi
		Summer Bibb	98.9c-k	17.2jk	5.2fgh	5.3bc	0.18gh
	based on plant needs	Red Sails	115.1c-g	20.3hi	5.4fg	4.2ef	0.126k-o
		Edox	126.6c-f	13.1l	3.4jkl	3.9fgh	0.13j-n
		Summer Bibb	254.4a	31.9b	4.5hi	2.9k	0.09pq
70 Red/ 30 Blue	Complete replacement	Red Sails	147.6bcd	22.6fg	4.8gh	1.8lmn	0.111-p
		Edox	16.8mno	13.5l	8.2d	3.0jk	0.111-p
		Summer Bibb	55.6g-o	28.1d	6.6e	4.1fg	0.16hij
	Based on EC	Red Sails	124.3c-f	18.3ij	5.6fg	3.5hij	0.111-p
		Edox	156.2bc	12.3lm	4.0ij	4.0fgh	0.15ijk
		Summer Bibb	85.3e-l	32.b	3.5jkl	3.4ijk	0.12k-p
	Based on plant needs	Red Sails	194.3b	19.8hi	5.3fgh	3.3ijk	0.17hi
		Edox	138.2b-e	12.8l	5.0gh	3.3ijk	0.13j-n
		Summer Bibb	290.1a	34.2a	5.9f	3.6ghi	0.14i-m
Blue	Complete replacement	Red Sails	35.9j-o	8.6op	1.96n	1.0o	0.07q
		Edox	13.9no	5.3qr	2.9lm	1.3no	0.11m-p
		Summer Bibb	32.8k-o	10no	3.8ijk	1.4mno	0.10m-p
	Based on EC	Red Sails	17.7mno	6.8pq	3.7i-l	1.5mno	0.10n-p
		Edox	15.9no	4.0r	3.4jkl	1.8lmn	0.13j-n
		Summer Bibb	34.5j-o	11lmn	3.7i-l	1.7lmn	0.111-p
	Based on plant needs	Red Sails	41.7i-o	5.4qr	3.0klm	1.9lm	0.13j-n
		Edox	24.0l-o	3.5r	3.0klm	1.7lmn	0.10op
		Summer Bibb	9.7o	9.6o	3.9ij	2.1l	0.143i-l

Based on the results, it was found that the E under white light treatment was higher than other treatments in all three replacement methods of nutrient solution and in all three lettuce varieties. Of course, it should be noted that in white light, the three lettuce varieties treated with replacement method of nutrient solution based on the plant needs had the highest transpiration rate (Table 5). But in this spectrum in the complete replacement method of the

nutrient solution and replacement based on EC, Summer Bibb and Edox improved the transpiration rate. In addition, in the conditions of monochromatic red light, Edox and Summer Bibb varieties in the condition of complete replacement of the nutrient solution, and all three lettuce varieties in the condition of replacement based on EC and Red Sails in the condition of replacement based on the plant needs caused an increase in E (Table 5). In the conditions of using the combination of red and blue light, Summer Bibb cultivar, Edox cultivar and Summer Bibb cultivar in conditions of complete replacement of nutrient solution, replacement based on EC and based on plant needs, respectively, increased the E (Table 5). Also, under monochromatic blue light, the E was significantly lower than other treatments (Table 5).

The results of the present study showed that white light application on three varieties of lettuce in all three replacement methods of nutrient solution caused an increase in the g_s (Table 5). Also, in this light spectrum, Summer Bibb and Red Sails varieties had the highest amount of stomatal conductance in the replacement method based on the plant needs (Table 5). Also, in the replacement method based on EC and the complete replacement of the nutrient solution, Edox cultivar improved the amount of g_s (Table 5). In other spectrums of light irradiated on the plants, much lower amounts of stomatal conductance than white LED light (Table 5). The use of Summer Bibb and Edox cultivars in the complete replacement method of nutrient solution and Red Sails and Summer Bibb in the replacement method based on EC under the conditions of using monochromatic red light caused an increase in g_s (Table 5). While the use of the combination of red and blue light and monochromatic blue light caused the lowest value of g_s (Table 5).

The results of the present study showed that the use of a combination of red and blue light and white light had the greatest effect on the intercellular CO_2 concentration of lettuce varieties in the condition of replacement method of the nutrient solution based on plant needs (Figure 2). It was also found that there was no significant difference between the red-light spectrum, the combination of blue and red and white LED light in the conditions of replacement based on EC in terms of intercellular CO_2 concentration (Figure 2). In addition, in the conditions of complete replacement of the nutrient solution, the use of red light and white light caused an increase in the content of intercellular CO_2 concentration (Figure 2). In all three replacement methods of the nutrient solution, the use of blue light causes a significant reduction in intercellular CO_2 concentration (Figure 2).

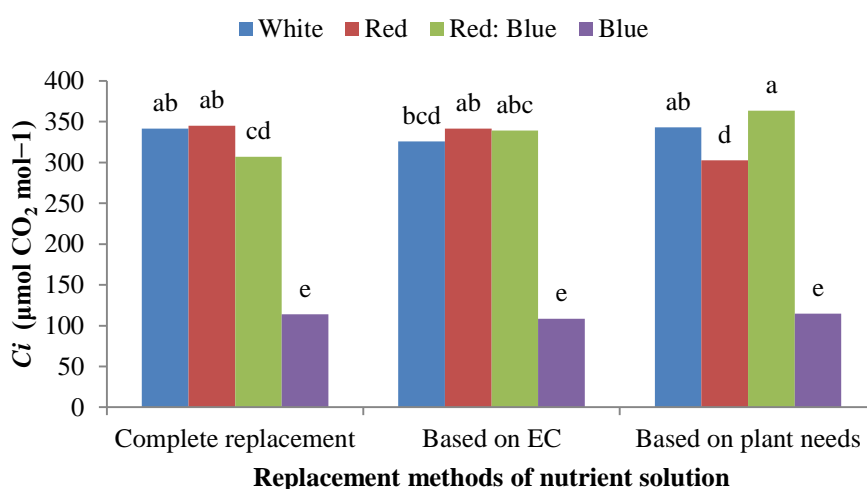


Figure 2. The interaction of different replacement methods of nutrient solution and different supplemental light spectrum on the C_i

4. Discussion

In the current research, it was found that supplementary white LED light and the combination of red and blue light produced the greatest effect on the fresh weight of shoots and roots of lettuce in all three replacement methods of nutrient solution. This positive effect can be related to the best role of light in the growth, development and reproduction of plants. On the other hand, light has the greatest effect on photosynthetic properties in plants in which plants perceive light through photoreceptors and convert it into carbohydrates. Eventually, these produced carbohydrates are transported to the overall plant structures by consumer organs such as roots and leaves for the better growth and development in plants. Researchers indicated that decreasing light intensity cause to reduce plant growth; fewer internodes resulted in shorter plants (Eskandarzade *et al.*, 2024). It has been shown that by the increase in the light intensity through the supplemental light, it is possible to accelerate the rate of growth and development of plants (6). In another study Malekzadeh *et al.* (2024) reported the positive effect of combination of red and blue light on the increase in fresh and dry weight of aerial organs and strawberry fruit. Improving the root structure increases the absorption of nutrients and their transfer to the aerial parts of the plants that result in the improved growth of the plants. Earlier researchers found that supplementing white light on lettuce caused an increase in the biomass of the plant due to the increase in photosynthetic properties (Zhang *et al.*, 2015; Chen *et al.*, 2016; Luković *et al.*, 2024). In another study by Liu *et al.* (2020), it was reported that the combination of red/blue light increased lettuce biomass. On the other hand, changing the nutrient solution in time and providing nutrients to plants during the growth period is an important factor for increasing the growth and reproduction characteristics of plants. In the present research, to reduce the consumption of water and nutrients, after conducting tests on the nutrient solution used, the concentration of elements in the new solution was adjusted and provided to the plants to prevent the accumulation of elements in the nutrient solution. Accumulation of nutrients in the root environment of plants causes salinity stress in plants that limits their ability to absorb water and nutrients. Therefore, for this purpose, nutrient solutions in this research were made available to plants as a suitable replacement for nutrients that prevented the salt stress around the roots and resulted in the lack of absorption of elements. In addition, the absorption of water and nutrients required by the different parts of the plant increased significantly due to the provision of suitable supplemental light conditions, thus resulted in an increase in the growth characteristics of the lettuce varieties under studied. Studies have shown that replenishment with the standard hydroponic solution resulted in an increased solution EC and concentrations of calcium, magnesium, and sulphate while decreasing nitrogen, phosphorus, and potassium. These findings support the idea that nutrient solutions should be changed or replaced with the proper nutrient elements after multiple rotations (Houston *et al.*, 2023). Scientists believed that photosynthetic function is among many factors that contribute directly to growth and biomass accumulation in plants and blue and red radiation of the visible spectrum play an important and direct role in these plant functions as they provide the energy needed for carbon assimilation. Additionally, both blue and red radiation also regulates many aspects of morphogenesis including shoot elongation, cell differentiation, modulating shoot growth and flower initiation and numerous biochemical and physiological processes including those involved in secondary metabolism (Hamm *et al.*, 2004; Kospell *et al.*, 2015).

The lighting system is among the most crucial elements for chlorophyll production. By regulating pigment synthesis, various light source wavelengths have an impact on distinct plant photoreceptors (Dougher and Bugbee, 1998). In low-light conditions (higher than minimum light intensity threshold that induce shade avoidance responses), plants increase their chlorophyll concentration to compensate for the lack of light (Beneragama and Goto, 2011). Therefore, an increase in chlorophyll concentration is an adaptive strategy to tolerate

light lowlight conditions. In the present study, the use of supplemental lighting resulted in an increase in photosynthetic pigments which was consistent with the researchers' findings on roses grown under controlled conditions under supplemental light treatment (Davarzani et al., 2023). Additionally, monochromatic red light may occur at intensities to initiate the process of chlorophyll production (Tripathy and Brwon, 1995). Ammozgar *et al.*, (2016) and chen *et al.*, (2106) reported that chlorophyll and carotenoid concentrations increased in the plants grown under combination of red and blue LEDs compared to those grown in the greenhouse that agreed with our results in this study. Also red monochromic LED light can increase the content of photosynthetic pigments (Chl a, Chl b, total chlorophyll, and carotenoids) (Lim *et al.*, 2023). It has been reported that the spectral composition of red LED matches with the red absorbance area of Chl a and b present in the chloroplasts of higher plants (Schoefs 2002), Nevertheless, it has been reported that blue light has complementary effect. In another study, optimum accumulation of chlorophyll a and chlorophyll b in *Triticum aestivum* plants was observed which were grown under red LEDs than those grown under blue LEDs (Dong *et al.*, 2014).

Our results showed that plants regenerated under white LED depicted higher P_N , which exactly corresponded to the g_s , C_i , and E . However, plants grown under combination of red and blue and monochromic red LEDs had middle amount of P_N , E and g_s compared to blue and white light sources. The lower P_N with the plants grown under blue LEDs could be due to the lower transpiration rate (E), and stomatal conductance (g_s). Nevertheless, the highest P_N was recorded with plants grown under white LED treatments. Our previous study on lettuce under different LED light treatments showed that the combination of red and blue light and monochromatic red light increased photosynthetic properties under floating culture conditions in a controlled environment (Soufi et al., 2024). 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. Because 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). Researchers believed that the gerbera plant grown under combination of red and blue light had higher internal C_i , P_N , g_s , and E , a finding that it is contrary to our findings in this research (Lim *et al.*, 2023). In our study, the Red and combination red and blue LED treatment had lower P_n , E and g_s values compared with the maximum P_n of white LED light. Miao *et al.* (2019) explained the phenomenon that monochromatic red light easily induces red light syndrome by long-term irradiation.

5. Conclusion

The present study was conducted to improve the growth and photosynthetic characteristics of lettuce varieties with different replacement methods of nutrient solution in the greenhouse during winter of 2020. The results indicated that white light, a combination of red and blue light, had the greatest effect on the growth characteristics of lettuce varieties and P_n , E and g_s compared to other light spectrums. But the combination of red and blue light and red light only improved and increased the photosynthetic pigments in lettuce varieties in different

replacement methods of nutrient solution. In this study, the use of replacement based on plant nutritional needs and then the complete replacement of the nutrient solution could increase the growth and photosynthetic characteristics of lettuce varieties. Reducing the consumption of water and nutrients through replacing the nutrient solution and manipulating light spectrum had a positive effect on the growth and development of lettuce varieties. As a recommendation, we suggest growing lettuce varieties by replacing the nutrient solution in aeroponics and using a combination of red and blue supplemental light for better commercial production.

Conflict of interest

The authors declares no conflict of interest

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