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Impact of light-emitting diode irradiation on photosynthesis, phytochemical composition and mineral element content of lettuce cv. Grizzly

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Abstract

Light-emitting diodes (LEDs) are a promising technology with a potential to improve the irradiance efficiency, light quality, and the light spectrum for increasing plant yield and quality. In this experiment, we investigated the impacts of various LED light qualities, including 100% red, 100% blue, 70% red + 30% blue, and 100% white, on the growth and photosynthesis, phytochemical contents, and mineral element concentrations in lettuce (*Lactuca sativa* L. cv. 'Grizzly') in comparison to normal greenhouse conditions. Photon flux of 300 μmol m^{-2} s^{-1} was provided for 14 h by 120 LEDs set on a 60 cm × 60 cm sheet of aluminum platform in the growth chambers, where plants were grown for 60 d. Fresh mass per plant was significantly higher when grown under 100% blue and 70% red + 30% blue LEDs compared to the other environments including greenhouse conditions. Phytochemical concentrations and a nutritive value of lettuce were also significantly affected by the light treatments. Chlorophyll and carotenoid concentrations increased in the plants grown under 70% red + 30% blue LEDs compared to those grown in the greenhouse. Vitamin C content was 2.25-fold higher in the plants grown under 100% blue LEDs compared to those grown in the greenhouse. Higher photosynthesis and maximal quantum yield of PSII photochemistry were also observed in the plants treated with LED lights. The application of LED light led to the elevated concentrations of macro- and micronutrients in lettuce possibly because of the direct effect of LED light and lower stress conditions in the growth chambers compared to the greenhouse. Although the mechanism of the changes in lettuce grown under LED is not well understood, the results of this study demonstrated that LED light could be used to enhance the growth and nutritional value of lettuce in indoor plant production facilities.

Additional key words: carotenoids; LED technology; lettuce; mineral content; phytochemicals.

Introduction

Light emitting diode (LED) is a unique type of diode consisting of a chip from semiconductor material doped with impurities to create a p–n junction leading to the monochrome light emission across the entire visible spectrum when electron current is passed through the semiconductor diode. They rapidly evolved from low-intensity signal indicators into powerful light sources used from street lighting to lighting of greenhouses and for urban agriculture, which is now a growing industry in high-tech greenhouses equipped with LED lights (Yeh and Chung 2009). Compared to the traditional and artificial light sources for photosynthetic organisms, LEDs have a very significantly longer life span, ease of light intensity control, and higher PAR efficiency which is ideal for irradiation of plants to revolutionize the production under controlled growth environments (Darko *et al.* 2014).

Recently, there is a growing concern on efficient energy usage, environmental impact of agricultural inputs, and food safety in production systems (Pinho *et al.* 2012). In reducing energy usage for crop production, LEDs are not only an efficient form of lighting because of their low energy consumption, but also generate low heat and as a consequence, less energy is used for cooling glasshouses, greenhouses, and plant factories (Watanabe 2009, Goto 2012). The monochrome nature of LEDs provides crops with specific and high quality irradiation with suitable wavelengths which means lesser energy consumption for growing crops. Moreover, the increased use of LEDs in environmentally controlled and

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Abbreviations: Car – carotenoids; Chl – chlorophyll; DM – dry mass; FM – fresh mass; F_v/F_m – maximal quantum yield of PSII photochemistry; LED – light emitting diode; MDS – multidimensional scaling; TCA – trichloroacetic acid.

closed-type plant production systems allows crop production to be continued throughout the year, regardless of external weather conditions (Schuerger *et al.* 1997, Darko *et al.* 2014). Therefore, it is predicted that LED lights become a light source with a considerable potential for high-power lighting in agricultural productions.

With the advent of LEDs, increasing research for commercial plant production utilizing LEDs has occurred. Studies have looked at the effects of different colors of LEDs and various combinations of fluorescent lights and LEDs on crop production. Some of the crops, which have been already cultured under LED light irradiation, are lettuce (Yanagi *et al.* 1996, Martineau *et al.* 2012), pepper (Brown *et al.* 1995), wheat (Goins *et al.* 1997), spinach (Yanagi and Okamoto 1997), and banana (Nhut *et al.* 2002). More recently, herbs, such as mint and basil, and pot flowers including petunia, primula, marigold, and stock were grown under LED irradiation with high marketability characteristics when they were compared with greenhouse or field-grown counterparts (Colquhoun *et al.* 2013, Sabzalian *et al.* 2014).

It is believed that the high fluence effect (light point intensity) of LEDs drives the accumulation of secondary metabolites through the stimulation of light receptors and possibly by triggering/inhibiting the expression of some regulatory genes (Liu *et al.* 2004, Tamulaitis *et al.* 2005). The two main receptors in plant cells, which work together to regulate the photomorphogenic responses, are phytochromes as the red light receptors and cryptochromes as the blue light receptors. The molecular mechanism of the signal transduction *via* cryptochromes and phytochromes is not clearly understood; however, it seems that they are involved in cellular protein-protein interactions. Therefore, the monochromatic lights could eventually lead to the altered localization of light-signaling proteins, changes in ion homeostasis, gene expression, and many other cellular activities (Lin 2002, Costa Galvão and Fankhauser 2015).

In lettuce, high power LED-lighting systems have been shown to accelerate the growth rate of plants compared to those grown under normal solar irradiance or under greenhouse hydroponic culture conditions (Chin *et al.* 2012, Martineau *et al.* 2012). Generally, blue and red LEDs are typically used for plant growth because chlorophyll (Chl) *a* and Chl *b* efficiently absorb wavelengths in blue and red regions. Johkan *et al.* (2010) found that in red leaf lettuce, red LEDs increased shoot fresh mass up to 25% after 17 d from sowing compared to fluorescent light. However, after 45 d from sowing, the highest fresh mass was obtained in lettuce plants treated with blue LED lights. In the other experiment, they showed that high intensity green LED lights were effective to promote lettuce growth compared to fluorescent light (Johkan *et al*. 2012). It is believed that red light irradiation without blue light is effective at stimulating the biomass accumulation of lettuce plants; however, red light alone induced abnormal leaf shape and had a negative effect on contents of polyphenolics and antioxidants in two lettuce cultivars (Son and Oh 2013). Nevertheless, Li and Kubota (2009) suggested both red and blue LEDs have a positive effect on the growth and development of another lettuce variety (*Lactuca sativa* L. cv. Red Cross).

Many studies have recently aimed to improve the nutritional value of lettuce using full or supplementary lighting with LEDs. Li and Kubota (2009) showed that cool fluorescent lighting supplemented with red LEDs increased the phenolic contents in red lettuce (cv. Red Cross) leaves. Zukauskas *et al.* (2011) reported that red LED lighting supplemental to high-pressure sodium (HPS) lamps increased the content of phenolic compounds in lettuce. High-intensity red LED light could also lower the nitrate content in lettuce, especially when applied at the terminal stage of plant growth (Samuolienė *et al.* 2009). It was later shown that the other monochromatic lights, such as cyan/green, also improved nutritional quality of different baby leaf lettuce varieties grown under HPS lights in the greenhouse (Samuolienė *et al.* 2012). Moreover, the addition of blue light to red light or an increase in blue light intensity increased total phenolic concentration and the antioxidant capacity of red leaf lettuce (Johkan *et al.* 2010, Son and Oh 2015). Blue light may activate the expression of chalcone synthase (CHS) and phenylalanine ammonia-lyase (PAL) genes, the key elements in the biosynthetic pathways of secondary metabolites (Jenkins 1997, Son *et al.* 2012). Therefore, it seems that supplemental blue and red light can be strategically employed to enhance many bioactive compounds and consequently the nutritional value of lettuce (Lin *et al.* 2013). However, the responses to different light wavelengths could be also cultivar dependent as species-specific influence of LED lights is being reported (Taulavuori *et al.* 2016).

Among the many genotypes of lettuce in the world, crisphead varieties are popularly cultivated under outdoor conditions in the United States and the Middle East while their responses to LED lighting under indoor conditions have not been investigated. Most studies on the plant responses to LED lighting only compare the growth and quality of the plant cultivated under different artificial light environments provided by the LEDs or a combination of LEDs and fluorescent or other light sources. However, LED-irradiated plant production in some regions of the world should be compared to the production under the traditional cultivations in the field or greenhouse in order to be acceptable by consumers. Also, as far as the nutritional values are concerned, the lettuce leaves are rich in minerals, antioxidants, and vitamin compounds. It is still unknown if LEDs may affect the uptake of nutrient elements by lettuce. The objectives of this study were, therefore, (*1*) to determine the effects of different LED-light sources on the growth, photosynthesis, and mineral element concentrations of the crisphead lettuce cv. Grizzly, and (*2*) to explore whether

LEDs provide sufficient light for production of lettuce with high quantity and quality in order to be competitive with lettuce produced in a traditional greenhouse under similar cultivation conditions.

Materials and methods

Plant material and experimental procedure: One month before the experiment, lettuce seeds (cv. Grizzly) were placed in each hole of 2.5 cm × 3.8 cm horticultural cubes containing cocopeat. The horticultural cubes were moistened with tap water and placed in plastic trays with clear covers. The trays were put on a greenhouse bench under ambient light conditions of 300 μmol(photon) m^{-2} s^{-1}, a relative humidity of 65 ± 10%, and an average temperature of 25 ± 2°C. At the beginning of the spring, when the seedlings had one set of fully expanded leaves, they were transferred individually to 1.9-L plastic pots containing a clay loam soil/cocopeat mixture (50%:50%). The pots, each containing one single plant, were placed in four types of LED-irradiated growth chamber located inside a laboratory and in the greenhouse (experimental greenhouse of Isfahan University of Technology, Isfahan, Iran, 32°44'N, 51°10'E; 1,591 m a.s.l.) in six biological replications. The growth chambers were fully isolated from the laboratory environment and had their separately set environmental parameters. Pots were irrigated daily with tap water and with the nutrient solution (1 g L^{-1}) containing the main nutrient elements (K, Mg, Ca, N, P) once a week (*Floral* 20-20-20; *Cifo SpA*, Bologna, Italy).

LED growth chamber and light control system: The LED growth chambers were designed and used as described by Sabzalian *et al.* (2014). Briefly, the control units were independently designed to support LED lighting in the four types of growth cabinets. In each cabinet, LED sheets containing 120 1W LEDs (0.25 A of input current, *Edison Opto*., Taiwan) emitting white (380–760 nm), red (650–665 nm), blue (460–475 nm) or red + blue (70%:30%) light were separately affixed to a ceramic and steel support to facilitate efficient heat transfer to the mounting substrate. Voltage to the arrays, which determines illumination intensities, was tuned *via* a self-made potentiometer to reach 300 μmol(photon) m^{-2} s^{-1} in each separate growth chamber at the leaf surface measured *via* a light meter (*LI-250A*, *LI-COR Inc*., USA). A microcontroller containing the control logic for setting the growth parameters was written in the assembly language and applied to each growth cabinet to adjust LED brightness. The other environmental parameters inside the growth chambers including temperature (25 ± 2°C), humidity (65 ± 10%), light intensity [300 μmol(photon) m^{-2} s^{-1}] and light/dark period duration (14/10 h) were set in the same way as those measured during the growth period in the greenhouse normally used for lettuce production.

Morphological data analysis: Lettuce plants were grown for two months under different light treatments including blue, red, red+blue (70%:30%) and white LEDs and greenhouse natural light conditions. After this period, fresh mass of shoots and roots were measured as plant biomass. Shoots and roots were dried in an oven for 48 h at 70°C and then weighed. The relative water content of each section was then calculated from the difference in fresh (FM) and dry masses (DM) as [(FM – DM)/FM] ×100 for each replication. Allometric coefficients were also calculated by the measurement of shoot to root ratio for both FM and DM obtained from the four LED growth chambers and greenhouse condition.

Evaluation of photosynthetic parameters: The CO_2 assimilation was measured by a portable photosynthesis system (*LCi*, *ADC Instruments*, UK). Leaf Chl fluorescence was also measured using *Handy PEA* (*Hansatech Instruments Ltd*., Norfolk, UK). The leaves were illuminated with saturating light and then after 15 min of adaptation to darkness, F_v/F_m was determined as: $[F_v/F_m = (F_m - F_0)/F_m]$, where F_m and F_0 are maximum and initial fluorescence yields of dark-adapted leaves, respectively. Chl *a* and *b* and total carotenoids (Car) were determined from 90% acetone extract of lettuce leaves using the formulas suggested by Lichtenthaler and Buschmann (2001) as follows:

$$\text{Chl } a \text{ [mg g}^{-1}] = \frac{(12.72 \times \text{OD663} - 2.59 \times \text{OD645})}{1000\text{W}} \times \text{V}$$

$$\text{Chl } b \text{ [mg g}^{-1}] = \frac{(22.88 \times \text{OD645} - 4.67 \times \text{OD663})}{1000\text{W}} \times \text{V}$$

$$\text{Car [mg g}^{-1}] = \frac{[(1000 \times \text{OD470} - 3.27 \times \text{Chl } a - 104 \times \text{Chl } b)/229]}{1000\text{W}} \times \text{V}$$

where V is the total volume of acetone extract [ml] and W is the fresh mass [g] of the sample.

Vitamin C and total phenolics: Vitamin C was determined by using indophenol method, the modified procedure outlined by Nielsen (2009). Briefly, a sample of 30 g of lettuce grown in each pot was ground using mortar and pestle with an addition of 10 ml of trichloroacetic acid (TCA). The mixture was further ground and strained and the extract was made up to 100 ml with the TCA mixture. A 10 ml of the TCA solution was pipetted into the 50-ml Erlenmeyer flask followed by 10 ml of the sample extract. The samples were titrated separately with the indophenol dye solution until a light rose pink persisted for 5 s. The amount of dye used in the titration was determined and used in the calculation of the vitamin C content. To obtain a standard curve, 0.05 g of ascorbic acid was mixed with TCA and

titrated as mentioned above. The total phenolic content of leaves was also determined in methanolic extracts from 1 g of FM by Folin-Ciocalteu method with gallic acid as spectrophotometric standard (Ragaee *et al.* 2006). Absorbance was obtained at 765 nm with a *DU-7* spectrophotometer (*Beckman*, USA).

Analysis of mineral elements: Dried tissues were ground to obtain homogenous samples. A 0.5 g of subsample was then dissolved in 10 ml of salicylic acid (SA)-H_2SO_4 mixture (Bremner and Mulvaney 1982). Aliquots were diluted with distilled water and analyzed for their K content by flame emission (*Flame Photometer 410*, Corning). The concentrations of Ca, Mg, Mn, Cu, Zn, and Fe were determined by atomic absorption spectroscopy (*Perkin-Elmer 800*, USA). Total nitrogen was determined by micro-Kjeldahl analysis (Helrich 1990) and phosphorus was measured using the Murphy and Riley reagents (Olsen and Sommers 1982).

Statistical analysis: All the data were analyzed using the *Statistical Analysis System (SAS Institute Inc*. 2004) program package according to a completely randomized design to compare different light conditions. After an analysis of variance (*ANOVA*), significant differences between means were determined by least significant difference (LSD) test ($p<0.05$).

Multidimensional scaling (MDS) analyses of the dissimilarity matrices of the five light environments (100% red, 100% blue, 70% red + 30% blue, 100% white, and greenhouse condition) were performed separately based on morphological, photosynthetic, and phytochemical and mineral element data sets using the proximity scaling algorithm (*PROXSCAL*, *SPSS 17.0; SPSS Inc*., Chicago, IL, USA). Principle components analysis (PCA) was also performed using *SPSS*.

Results and discussion

Impact of LED irradiation on lettuce growth: The lettuce plants (*Lactuca sativa* L. cv. Grizzly) grown under blue + red LEDs were visually better than those grown in the greenhouse or under the other LED combinations (Fig. 1*A*). The lettuce head was also shaped better when grown under blue + red and blue LEDs. In contrast, under pure red LEDs, leaves were not fully expanded and crispy, stem was fragile, and the head was not formed, indicating that pure red light was not adequate to complete leaf expansion and support a tight head formation in the crisphead lettuce (Fig. 1*B*). Chen *et al.* (2014) also reported that 'Green Oak Leaf' lettuce showed sparse and fragile appearance under monochromic red irradiation at the seedling stage. However, during the later growth period, lettuce became vigorous in their study, which was not observed during the long duration of our experiment. Indeed, the crisp-head lettuce showed excessive hypocotyl elongation and fragile shoot under monochromic red light. Also, it seems that blue light is not only important for leaf expansion (Johkan *et al.* 2012) but also for leaf positioning and rotation.

The plants grown under blue + red LED irradiation had significantly higher shoot and root FM, although no significant difference was found between blue + red and blue LED-grown plants regarding the lettuce FM (Table 1). In contrast, shoot and root DM were greater under the greenhouse conditions than those obtained from the LED-growth chambers (Table 1). Okamoto *et*

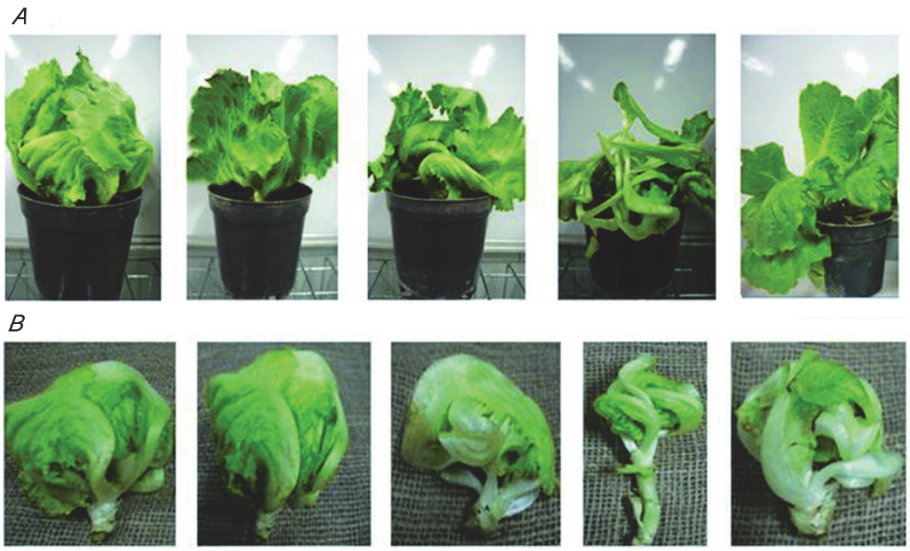

Fig. 1. Left to right: lettuce grown under blue + red, blue, white, and red LEDs and in the greenhouse (*A*). The tight head of crisp leaves shaped under different LED lights and in the greenhouse (*B*).

Table 1. Mean (± SE) comparison of morphological traits and shoot and root water contents measured on lettuce (cv. Grizzly) irradiated under different light sources. Values represent means of six replicates. FM – fresh mass; DM – dry mass.

Light source	Shoot FM [g]	Root FM [g]	Shoot DM [g]	Root DM [g]	Shoot to root FM ratio	Shoot to root DM ratio	Shoot water content [%]	Root water content [%]
Greenhouse	33.69 ± 0.73^{b}	29.33 ± 1.76^{b}	8.42 ± 0.08^{a}	3.49 ± 0.17^{a}	2.39 ± 0.26^{c}	2.44 ± 0.24^{b}	77.84 ± 0.20^{c}	88.07 ± 0.32^{d}
Blue LED	128.79 ± 2.69^{a}	23.83 ± 1.36^{c}	3.63 ± 0.16^{c}	1.18 ± 0.01^{c}	5.43 ± 0.28^{b}	3.11 ± 0.23^{b}	97.18 ± 0.08^{a}	95.01 ± 0.30^{b}
Red LED	22.10 ± 1.31^{c}	1.67 ± 0.16^{d}	0.62 ± 0.03^{d}	0.05 ± 0.01^{d}	16.54 ± 0.58^{a}	11.87 ± 0.55^{a}	97.17 ± 0.10^{a}	96.55 ± 0.62^{a}
Blue + red LED	128.45 ± 1.93^{a}	36.33 ± 0.92^{a}	5.99 ± 0.29^{b}	2.50 ± 0.14^{b}	3.53 ± 0.20^{c}	2.51 ± 0.21^{b}	95.32 ± 0.27^{b}	93.11 ± 0.34^{c}
White LED	129.31 ± 0.99^{a}	21.67 ± 0.83^{c}	3.41 ± 0.11^{c}	1.49 ± 0.15^{c}	6.03 ± 0.44^{b}	2.32 ± 0.07^{b}	97.36 ± 0.09^{a}	93.08 ± 0.67^{c}

al. (1997) found that lettuce had the maximum whole plant DM under a combination of red and blue LEDs compared to pure red or blue LED lights; however, they did not evaluate control plants grown in a normal greenhouse. The white LED light resulted in the same shoot FM, but lower DM than blue + red LEDs (Table 1). White light is composed of a combination of red and blue lights and other light wavelengths which are not photosynthetically active compared to red and blue. This may diminish the effect of white light on the net photosynthesis and synthesis of metabolites compared to the combination of red and blue lights (Sabzalian *et al.* 2014).

The highest ratio of shoot FM and DM to the corresponding masses in root was observed in the plants irradiated by red LEDs (Table 1). However, this was achieved due to a very low root biomass in the absence of blue light, *i.e.* in the growth chamber with pure red LED (Table 1). This may indicate that pure red LED light was not adequate to complete the full growth of lettuce (cv. Grizzly) and although red light may contribute more to the plant photosynthesis than blue light (see the section below), it was not correlated with the increase in FM and DM of lettuce. As indicated in Table 1, the ratio of shoot to root FM and shoot and root water contents of plants grown in all LED-irradiated cabinets were significantly higher than those grown under the greenhouse conditions. It might suggest that the plants grown in LED growth chambers were not exposed to water shortage, while those experiencing greenhouse conditions might be affected by water stress possibly due to light fluctuations and/or solar infrared irradiation.

Pigments and photosynthetic parameters: Plants grown under blue + red LED irradiation had significantly higher content of Chl *a* and *b* and Car. However, the highest ratio of Chl *a/b* and the maximum capacity of photosynthesis were observed in the plants irradiated with red LEDs (Table 2). It has been reported that the spectral composition of red LED matches with the absorbance area of Chl *a* and *b* in the chloroplasts of higher plants (Schoefs 2002, Wang *et al.* 2007). Nevertheless, it has been reported that blue light has a complementary effect. For instance, Brown *et al.* (1995) compared pepper (*Capsicum annuum* L.) plants grown under red LED with similar plants grown under red LED plus blue light emitted from fluorescent lamps. Pepper biomass of shoots and roots was reduced when grown under red LED without blue wavelengths compared to plants grown under supplemental blue light. Although the significance of red light on plant photosynthesis is evident, it is believed that at least in some species, plants could not terminate their normal growth under pure red LEDs (Yorio *et al.* 2001). The conclusion of this study and similar literature concerning the lower effect of red LED compared to blue on root and shoot growth is in contrast to some other reports showing red light-promoted rooting (Wu and Lin 2012) and increased shoot biomass and essential oil content in some plant species (Sabzalian *et al.* 2014). Also, contrary to the previous reports, in the present study it seemed that red light had complementary effect on blue light, since blue + red LEDs increased the DM of lettuce compared to the pure blue LEDs (Table 1).

There are also a considerable number of studies indicating that Chl and Car synthesis and content were higher in lettuce and other plants treated with blue LEDs compared to the other lights (Tanaka *et al.* 1998, Johkan *et al.* 2010, Markou 2014). This was not evident in our study; blue + red complementary showed the strongest effect in the induction of both Chl *a* and *b* and Car synthesis in leaves of lettuce.

Higher accumulation of these pigments could lead to the higher light absorption, elimination of light-induced reactive oxygen species, and consequently improved shoot growth (Johkan *et al.* 2010). Higher content of water may also justify why the quantum efficiency of Chl, *i.e.*, F_v/F_m, was significantly higher in the plants grown in LED growth chambers than those kept in the greenhouse (Table 2).

Table 2. Mean (± SE) comparison of photosynthetic and phytochemical traits measured on lettuce (cv. Grizzly) irradiated under different light sources. Values represent means of six replicates. Means followed with *the same letter* in each column are not significantly different ($p<0.05$). Chl – chlorophyll; Car – carotenoid; P_N – net photosynthetic rate; F_v/F_m – maximal quantum yield of PSII photochemistry.

Light source	Chl *a* [mg g^{-1}]	Chl *b* [mg g^{-1}]	Chl *a*+*b* [mg g^{-1}]	Chl *a*/*b*	Car [mg g^{-1}]	P_N [μmol(CO_2) m^{-2} s^{-1}]	F_v/F_m	Total phenolics [mg g^{-1}]	Vitamin C [mg 100 g^{-1}]
Control	0.29 ± 0.02^{c}	0.12 ± 0.01^{b}	0.41 ± 0.02^{b}	2.47 ± 0.05^{a}	0.22 ± 0.01^{bc}	3.93 ± 0.96^{d}	0.78 ± 0.004^{b}	0.21 ± 0.02^{b}	3.40 ± 0.92^{d}
Blue LED	0.18 ± 0.02^{d}	0.12 ± 0.01^{b}	0.29 ± 0.01^{c}	1.58 ± 0.20^{c}	0.21 ± 0.01^{c}	16.22 ± 0.67^{b}	0.83 ± 0.005^{a}	0.35 ± 0.02^{b}	11.05 ± 2.69^{a}
Red LED	0.35 ± 0.02^{b}	0.13 ± 0.005^{b}	0.48 ± 0.02^{b}	2.69 ± 0.01^{a}	0.24 ± 0.01^{bc}	20.66 ± 1.49^{a}	0.80 ± 0.01^{ab}	0.23 ± 0.07^{b}	7.91 ± 0.85^{b}
Blue + red LED	0.71 ± 0.01^{a}	0.50 ± 0.04^{a}	1.22 ± 0.04^{a}	1.43 ± 0.08^{c}	0.59 ± 0.05^{a}	15.33 ± 1.32^{b}	0.81 ± 0.01^{ab}	0.23 ± 0.07^{b}	6.87 ± 0.52^{b}
White LED	0.28 ± 0.01^{c}	0.14 ± 0.01^{b}	0.42 ± 0.01^{b}	2.01 ± 0.02^{b}	0.29 ± 0.001^{b}	11.93 ± 2.38^{c}	0.81 ± 0.008^{ab}	1.18 ± 0.06^{a}	4.65 ± 0.66^{c}

Table 3. Mean (± SE) comparison of mineral element concentrations in lettuce (cv. Grizzly) irradiated under different light sources. Values represent means of six replicates. Means followed with *the same letter* in each column are not significantly different ($p<0.05$).

Light source	N [g 100g^{-1}]	K [g 100g^{-1}]	Ca [g 100g^{-1}]	Mg [g 100g^{-1}]	P [g 100g^{-1}]	Fe [mg kg^{-1}]	Zn [mg kg^{-1}]	Cu [mg kg^{-1}]	Mn [mg kg^{-1}]
Control	1.23 ± 0.04^{c}	2.60 ± 0.04^{c}	0.7 2 ± 0.08^{c}	0.23 ± 0.01^{c}	0.17 ± 0.005^{e}	218.34 ± 12.88^{e}	27.87 ± 2.68^{c}	13.33 ± 1.05^{c}	20.23 ± 0.04^{c}
Blue LED	2.94 ± 0.01^{b}	4.16 ± 0.01^{b}	2.75 ± 0.04^{ab}	0.80 ± 0.03^{a}	0.27 ± 0.01^{d}	334.78 ± 7.28^{d}	52.65 ± 1.90^{a}	17.33 ± 1.04^{b}	52.59 ± 0.95^{b}
Red LED	2.83 ± 0.25^{b}	5.24 ± 0.30^{a}	2.52 ± 0.17^{b}	0.67 ± 0.03^{b}	0.48 ± 0.03^{b}	887.37 ± 4.66^{a}	52.91 ± 0.40^{a}	28.67 ± 1.21^{a}	84.95 ± 2.13^{a}
Blue + red LED	3.44 ± 0.29^{a}	3.54 ± 0.29^{b}	2.52 ± 0.02^{b}	0.85 ± 0.005^{a}	0.40 ± 0.02^{c}	436.68 ± 14.56^{c}	35.62 ± 1.60^{b}	18.00 ± 0.88^{b}	52.59 ± 3.50^{b}
White LED	3.51 ± 0.19^{a}	5.37 ± 0.19^{a}	2.95 ± 0.04^{a}	0.87 ± 0.005^{a}	0.58 ± 0.03^{a}	494.90 ± 25.74^{b}	52.65 ± 3.09^{a}	20.67 ± 2.44^{b}	56.63 ± 1.90^{b}

Vitamin C and total phenolics: The results indicated that blue LED light was significantly effective to increase the vitamin C content of lettuce. On the contrary, the content of vitamin C in the plants grown under greenhouse conditions was the lowest (Table 2). The content of phenolics was also slightly higher (but it was not significant) in the lettuce grown under blue compared to red and blue + red LEDs; however, the values for the white light was significantly higher.

Vitamin C and phenolics are considered as potent water-soluble antioxidants and this study indicated that blue LED light could manipulate plant metabolism and improve the antioxidant properties of lettuce. Johkan *et al.* (2010) concluded that the antioxidant activities of lettuce seedlings treated with blue LED lights were higher than those grown under fluorescent and red lights. Blue light could also increase vitamin C content in immature strawberries when they were placed under LED irradiation for four days (Kim *et al.* 2011).

It is believed that blue light may affect transcription and expression of many genes involved in metabolic pathways throught direct signal transduction from a photoreceptor to transcriptional regulators. For instance, Imaizumi *et al.* (2000) reported the mRNA level of *CRY5* gene (the cryptochrome gene) in fern, *Adiantum capillus-veneris*, irradiated with blue light increased up to 300–400 fold. In contrast, *Arabidopsis CRY1* expression is not obviously regulated by blue light; however, it acts as a blue light receptor to regulate light induction and expression of chalcone synthase, which is the key enzyme of the flavonoid/phenolics biosynthesis pathway (Jenkins 1997). Wojciechowska *et al.* (2015) found significant correlation between the content of vitamin C and soluble sugars in lamb's lettuce (*Valerianella locusta*) leaves. Therefore, it seems that blue light is not only involved in the regulation of vitamin C synthesis through its impact on the blue light receptors and phytochromes, but also *via* the increase in the rate of photosynthesis and the formation of sugars.

Under natural and greenhouse conditions, plant growth could be seriously suppressed by the environmental stresses as a consequence of the overproduction of reactive oxygen species (Moran *et al.* 1994). Indeed, plants with better environmental stress tolerance have generally higher antioxidant capacity under stress conditions when compared to plants without stress tolerance (Kasuga *et al.* 1999, Roxas *et al.* 2000). Blue and red lights supplementary share some mechanisms to stimulate the plant's defense system and increase the biosynthesis of phenolic compounds in plants (Taulavuori *et al.* 2016). Therefore, plants irradiated with LED light and having a higher content of antioxidant compounds may possess higher tolerance if subsequently exposed to environmental stresses. This should be further investigated.

Mineral element contents: Our results showed that the lettuce grown under red LED light had significantly higher concentrations of K, Fe, Zn, Cu, and Mn. N and Mg contents were significantly higher in the plants grown under blue + red and white LEDs irradiation. The highest concentration of Ca was obtained from the plants grown under blue and white LEDs. P accumulation was significantly higher in lettuce grown under white LED irradiance (Table 3). Our results of element analysis demonstrated that the plants grown under different LED lights accumulated dramatically higher concentrations of mineral elements compared to those grown in the greenhouse. As an example, N and Mg concentrations significantly increased to 1.79 and 2.69 times in the lettuce grown under blue + red LEDs compared to the greenhouse conditions (Table 3).

Interestingly, the principle components analysis (PCA), which was performed in order to understand the relation between the photosynthetic rate (P_N) and other variables, showed high colinearity between photosynthesis and mineral element contents (Table 4). A positive relation was also found between the P_N and F_v/F_m and shoot and

Table 4. The colinearity among lettuce characteristics based on the first two components of PCA analysis. P_N – net photosynthetic rate; SFM – shoot fresh mass; RFM – root fresh mass; SDM – shoot dry mass; RDM – root dry mass; SRFMR – shoot to root fresh mass ratio; SRDMR – shoot to root dry mass ratio; SWC – shoot water content; RWC - root water content; Chl – chlorophyll; Car – carotenoid; F_v/F_m – maximal quantum yield of PSII photochemistry; Phenol – phenolics content; Vit C – vitamin C content.

Parameter	Rotated components	
	1	2
P_N	0.912	0.069
SFM	0.224	0.836
RFM	–0.647	0.750
SDM	–0.956	0.291
RDM	–0.942	0.312
SRFMR	0.760	-0.570
SRDMR	0.575	-0.618
SWC	0.942	0.322
RWC	0.961	0.004
Chl *a*	–0.036	0.659
Chl *b*	–0.040	0.835
Chl (*a*+*b*)	–0.041	0.742
Chl *a*/*b*	–0.065	-0.923
Car	0.006	0.843
F_v/F_m	0.627	0.491
Phenol	0.296	0.116
Vit C	0.593	0.136
N	0.784	0.589
K	0.911	-0.175
Ca	0.897	0.386
Fe	0.782	0.597
Zn	0.760	0.126
Cu	0.790	-0.348
Mn	0.908	-0.170
Mg	0.845	-0.360
P	0.950	-0.135

root water contents. However, the biplot showing the measured variables based on the first two principle components indicated no colinearity between the photosynthetic activity with shoot and root FM and DM and photosynthetic pigments (Fig. 2). Sabzalian *et al.* (2014) also found no particular trend between the photosynthetic activity and DM of mint (*Mentha* spp.) grown under LED lights. This may suggest that the strategy in utilization of fixed CO_2 and the factors affecting the P_N of lettuce are different and depend on the light source. Instead, there was correlation between the P_N and shoot to root FM and DM ratios indicating that under LED lights, more photosynthetic assimilates were probably redirected to the shoots instead of roots.

Generally, higher element concentrations in the lettuce grown in LED-growth chambers might be related to lower or no stress condition (*i.e.,* irradiance fluctuations) inside the growth cabinets compared to the greenhouse. This could be also inferred from the high colinearity between mineral element concentrations and shoot and root water contents (Table 4). However, for plants grown under different LED lights, there is little information on the stimulation of mineral element accumulation. Kopsell and Sams (2013) reported that a short duration of blue light before harvest significantly increased a nutritional value of broccoli microgreens by increasing the absorption of some macro- and micro-elements. Shin *et al.* (2013) also showed that concentrations of Ca, Mg, Mn, and Fe were higher in lettuce (cv. Chung Chi Ma) grown under blue or red + blue LEDs compared to the other light environments. Therefore, it is possible that LED lights could increase fertilizer uptake and allow the fertilizer to be used more efficiently in plant production.

It has been well established that light quality constitutes signals that can trigger metabolic modifications (Liu *et al.* 2004). Red LED may affect the metabolic pathways of plants and presumably water absorption leading to an increase in mineral element contents in leaves. However, LEDs may also alter mechanisms intervening in active absorption of elements. There is evidence that blue light triggers the opening of ion channels located on cell plasma membranes. This blue-light signaling not only promotes the efflux of some mineral elements, such as calcium, in the cytosol but also plays a critical role in the signaling process of cryptochromes (Lin 2002). Subsequently, cryptochromes as the blue-light receptors harness blue-light energy in order to increase the signaling of higher uptake of some macro and micronutrients (Jing 2009).

Tamulaitis *et al.* (2005) showed that LED lights were able to change the secretion of plant phytohormones, particularly GA_3. This may lead to the improvement in morphogenesis and productivity of the plant which contributes to the better nutrient and mineral uptake. These effects are warrant of further investigations because of their impact on the nutritional value of plants grown under LED lighting.

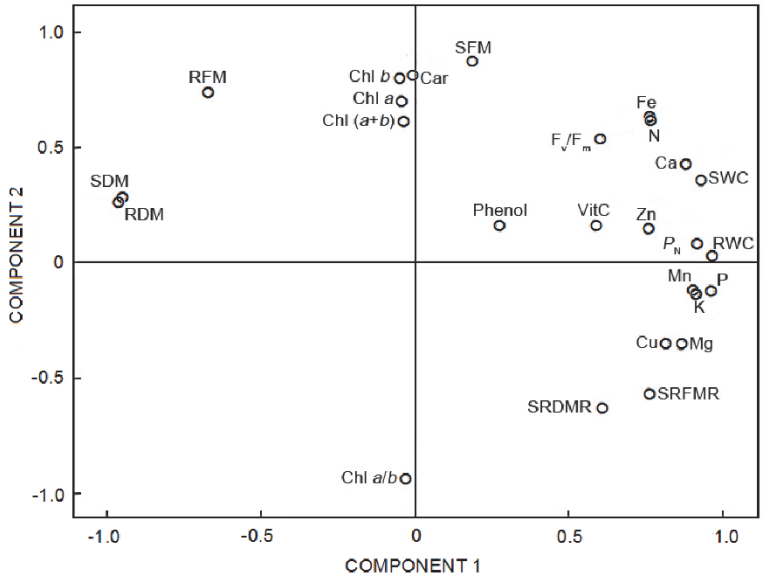

Fig. 2. The plot of principle component analysis showing the relation between the photosynthetic rate and other characteristics measured on lettuce grown under different light environments. P_N – net photosynthetic rate; SFM – shoot fresh mass; RFM – root fresh mass; SDM – shoot dry mass; RDM – root dry mass; SRFMR – shoot to root fresh mass ratio; SRDMR – shoot to root dry mass ratio; SWC – shoot water content; RWC – root water content; Chl – chlorophyll; Car – carotenoid; F_v/F_m – maximal quantum yield of PSII photochemistry; Phenol – phenolics content; Vit C – vitamin C content.

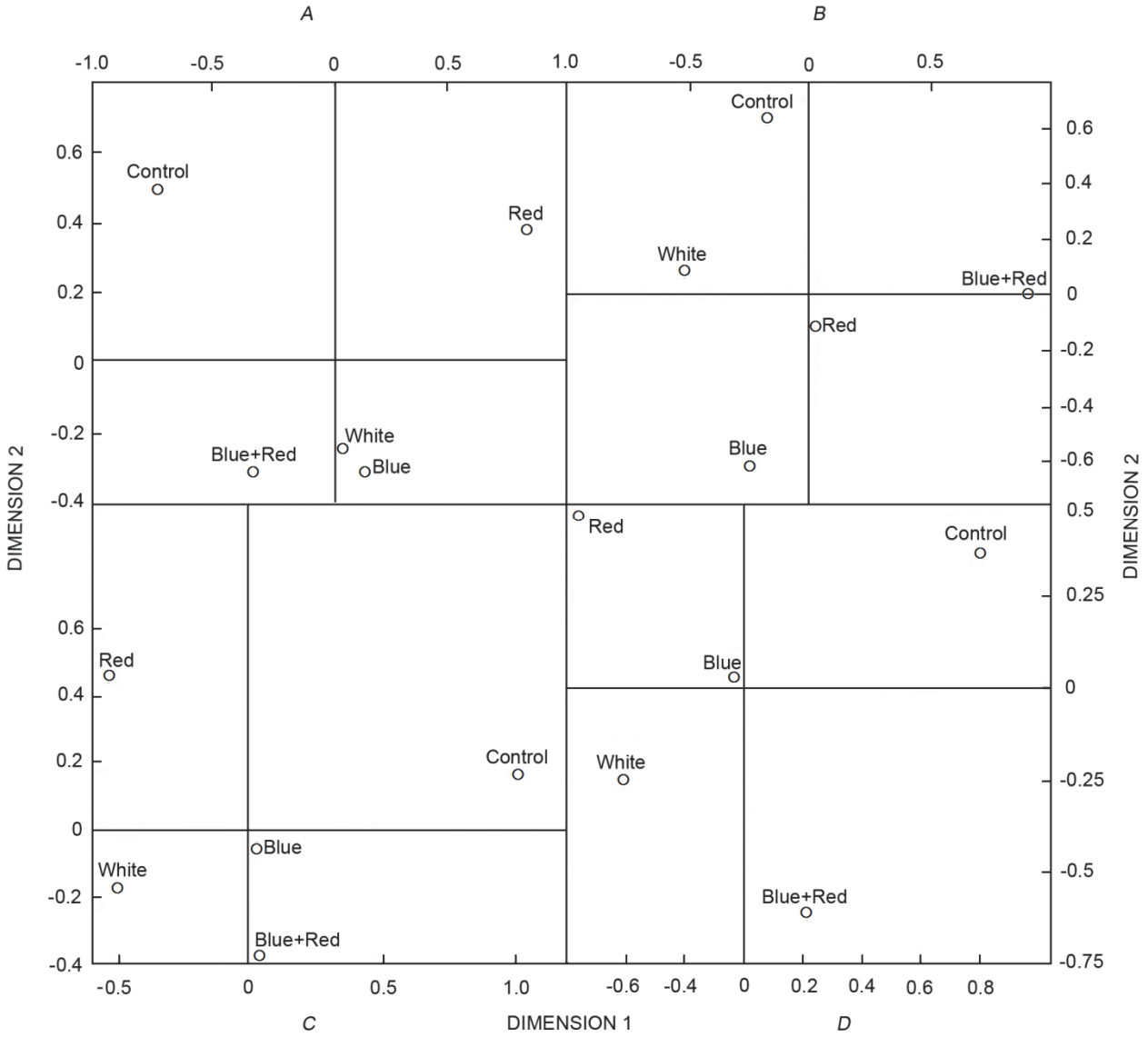

Fig. 3. Two-dimensional figures showing the proximity of the five environmental conditions based on multidimensional scaling using the *PROXSCAL* algorithm. Configurations were drawn separately for morphological (*A*), photosynthetic and phytochemical (*B*), mineral elements (*C*), and total data sets (*D*).

Two-dimensional distribution of light environments: The multidimensional scaling using different data sets separated the light environments into distinct groups (Fig. 3). Based on the morphological data, blue, blue + red, and white LED environments were grouped as a clump at −0.5 to 0.5 in dimension 1 and −0.4 to −0.2 at dimension 2. Red LED and greenhouse control environments were discriminated from this group at dimension 2 and were separated from each other at dimension 1 (Fig. 3*A*). In contrast, using the photosynthetic and phytochemical data set, blue + red LED environment was fully separated from the other environments at dimension 1 and blue LED and greenhouse environments showed separation at dimension 2 (Fig. 3*B*). Based on the data set for elements, blue, blue + red, and white LED environments were grouped together at an orientation of −0.4–0.0 at dimension 1 and −0.4 to 0.0 at dimension 2 (Fig. 3*C*). Using all the measured data, two-dimensional scaling showed almost full separation of all environments. Blue + red LED environment was separated from red LED and control environments at dimensions 1 and 2 and from white and blue LED environments at dimension 2 (Fig. 3*D*). These grouping patterns confirmed that the application of different LEDs largely changed morphological, photosynthetic, and phytochemical status of lettuce. On the other hand, white, blue, and blue + red LED light environments showed more similar effect on the morphological traits variation and mineral element concentrations than that on the photosynthetic and phytochemical characteristics. We concluded that these

LED lights could be possibly used for lettuce (cv. Grizzly) cultivation in the closed environments inter-changeably. The effect of red LED light and control environment (greenhouse) on the lettuce characteristics seemed to be distinctive from the other light conditions.

Conclusion: Our experiment showed the higher fresh mass production of lettuce in response to the irradiation by pure blue or 70% red + 30% blue LEDs compared to the other environments including the normal greenhouse conditions. However, differences in the growth could not be associated with the plant photosynthetic capacity. Based on the higher content of vitamin C and phenolics, it seemed that lettuce plants irradiated with LED light possessed higher antioxidant properties. Concentrations of macro and micro nutrients increased in the lettuce grown under LED lights compared to the greenhouse condition. This may also improve the nutritional value and quality of lettuce. Understanding of the involved mechanisms with which LED light could trigger higher synthesis of phytochemicals and higher uptake of minerals may extend the application of LED lights for improving the quality and economic yield of plant productions in controlled closed environments.

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