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Research article

Investigation of photosynthetic effects, carbohydrate and starch content in cress (*Lepidium sativum*) under the influence of blue and red spectrum

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ABSTRACT

In order to study the effect of the quality of different LED light spectra (90%R+10%B, 60%R+40%B and control) on photosynthetic parameters (photosynthetic rate (PG), Fv/Fm and ΦPSII) of stomatal conductance, transpiration rate, carbohydrate, starch and chlorophyll index on cress (*Lepidium Sativum*), a pot experiment was conducted under the greenhouse cultivation-without-soil (hydroponics) condition in the form of split plot based on a completely randomized design with 6 replications. The results showed that the combined application of blue and red light spectra with different percentages had a positive and significant effect on all traits. The highest amounts of each of the photosynthetic parameters in the 60R:40B treatment were 12.4, 0.87, and 0.92 (μmol CO_2 m^{-2} s^{-1}), respectively, and the lowest amounts (19.6, 0.39, and 0.44 (μmol CO_2 m^{-2} s^{-1})) were observed in the control treatment. The highest amounts of stomatal conductance, carbohydrate and starch of leaves which were 0.3 ($cm.s^{-2}$), 5.59 and 6.44 (mg.g-1 FW), respectively, were observed in the 90R: 10B treatment as a result of red light increase. Furthermore, in the control treatment, the light source of which was the natural sunlight, the lowest amounts of 0.11 ($cm.s^{-2}$), 1.98 and 1.09 ($mg.g^{-1}$ FW) were observed. The highest transpiration rate (25/83 ($mol.m^{-2}.s^{-1}$)) was observed in the 60R: 40B treatment which had experienced a significant increase compared to the control light (sunlight) treatment and the lowest transpiration rate (5.5 ($mol.m^{-2}.s^{-1}$)) was in the control (sunlight) treatment. The chlorophyll index in the 60R: 40B treatment was 41.18, which showed a significant difference from the other treatments ($p \leq 0.01$) and the lowest amount of 25.5 was detected in the control treatment. As a result, it can be stated that the use of blue and red light spectra in combination with different percentages can have various positive effects on the growth and development of plants; therefore, the existence of both types of spectra is suggested. This technology means that a particular combination of LED light spectra can be useful for a variety of commercial greenhouse products, especially the valuable ones.

1. Introduction

Cress (*Lepidium sativum*) is an annual plant which is herbaceous and comes from the Crucifera family. To grow and develop, this plant requires optimal levels of CO_2, light, nutrients, temperature, and water. Cress is both edible and medicinal because of it being rich with minerals and vitamins A and C and as it can be of great use in the treatment of anemia and blood purification (Yamori et al., 2010).

Adjusting light wavelengths can control and optimize processes such as photosynthesis, germination, flowering, and biomass accumulation (Pinho, 2008; Vänninen et al., 2010; Yeh and Chung, 2009).The light influences green plants growth and it can limit their productivity in case it is too excessive or week. Oxygen radicals can be generated and photoinhibition occurs if it is too excessive, and etiolation symptoms appear and photosynthesis cannot work efficiently if it is too week (Solymosi and Schoefs, 2010).Light intensity and quality can also affect plant development and growth and can lead to different susceptibilities to photoinhibition (Macedo et al., 2011). For instance, an increase in photosynthetic carbonfixation is detected at high light intensity; however, when a plant is exposed to excess light, the chloroplast lumen becomes acidic in nature, which leads to the electron transport chain reduction and the accumulation of excitation energy within the chloroplast. This may result in the generation of reactive oxygen species (ROS), namely, superoxide and hydrogen peroxide. ROS accumulation can cause lipid peroxidation and enzymatic antioxidants reduced accumulation, existing as plants defense system (Asada, 1999). Zuchi and Astolfi (2012) observed some species that were cultured under high CO_2 concentration and high photosynthetic photon flux (PPF) to have high photosynthetic

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flux. Plants are consistently competing with each other over gaining light as they are stationary and this competition can result in some changes in their morphology and growth. This fact could lead to understanding that quantity, quality and photoperiod modulation can also influence plant development and growth (Vänninen et al., 2010).

The light receptors in plants have the ability of sensing signals of light intensity and direction, and there are three major groups of them including cryptochromes, sensitive to UV-A and blue light; phytochromes, sensitive to red light and far red (Cashmore et al., 1999); and phototropins (Briggs and Huala, 1999). Red and blue light wavelengths can regulate plant development. Red light (660 nm) is absorbed by chlorophyll and carotenoids which are photosynthetic pigments and also creates a photostationary state in the absence of far-red or darkness dominated by the far-red form of phytochrome (Pfr) by stimulating the plant photoreceptor phytochrome. Blue light (400–500 nm) can affect stomatal opening, stem elongation, and phototropism and also its photoreceptor families together with phytochrome control processes in plants such as circadian rhythm and de-etiolation (Massa et al., 2008). Based on the studies conducted by Brown et al. (1995), LEDs can be better tools for creating controlled environments for plant cultivation than fluorescent lamps as they consume very low energy, almost generate no heat, have tailored spectrum and last plenty of years (Tamulaitis et al., 2005). Schoefs (2002) applied LED-based illumination to some species of plants on plant growth in vitro to examine the illumination spectrum and photon flux density (PFD) effects. "The efficiency of 650–665nm wavelengths of red LEDs on plant growth is fit with the absorption peak of chlorophylls and phytochrome"; whereas the blue light indicated that red and blue LEDs can imitate natural light for plant growth. In addition, the combination of red and blue lights showed a higher photosynthetic activity in comparison with monochromatic lights (Sabzalian et al., 2014). Stomatal conductance and photosynthetic rates in wheat were shown to be higher under red-LED light supplemented with blue light. It was proposed that the enhancement of photosynthetic rate by increased stomatal conductance can be associated with the increase in dry matter accumulation under the abovementioned condition (Goins et al., 1997). Nevertheless, it was reported that although stomatal opening was stimulated, increase in photosynthesis in eaves of lettuce was not detected in this condition (Yorio et al., 2001). Consequently, it is still not clear if dry matter productivity and leaf photosynthesis of every plant species are affected by blue light. There are also studies that demonstrate blue light can have an effect on leaves photosynthesis biochemical properties. Based on the study carried out by Senger and Bauer (1987), higher Chl a/b ratios were observed in plants grown under blue fluorescent lamps, smaller amounts of light-harvesting Chl a/b-binding protein of PSII (LHCII) per unit Chl content (Leong and Anderson, 1984) than plants are grown under red fluorescent lamps. However, it remains unclear whether the gas exchange between the leaves of the plants that were grown under red light with or without supplemental blue light are because of the changes in the photosynthesis biochemical properties or not. Actually, phytochromes are more sensitive to red than to blue; while, cryptochromes and phototropins are blue light-sensitive (Whitelam and Halliday, 2007).

When combinations of red and blue LED lights are used, blue light enhancement effect is greater on photosynthetic capacity in comparison with the time blue color of broad-band light is made deficient by a filter (Matsuda et al., 2008). As a matter of fact, little is known about the type of blue light enhancement effect on leaf photosynthetic capacity; whether it is a quantitative progressive response, a qualitative threshold response or a combination of both. There are a few quantitative blue light responses identified in leaves that are mentioned by (Jarillo et al., 2001) which are chloroplast movement and stomatal conductance. A greater photosynthetic capacity was discovered by Matsuda et al. (2008) for spinach leaves that were grown under 300 μmol m^{-2} s^{-1} mixed red/blue irradiance containing 30 μmol m^{-2} s^{-1} blue compared to the leaves grown under red alone. Study have shown that red light has an increasing effect on plants photosynthetic product accumulation; nevertheless, supplementing red with blue light could result in higher accumulation of these compounds (Zheng et al., 2010). According to (Goins et al., 1997), the reason was the coincidence of red and blue spectral energy distribution with the chlorophyll absorption spectrum, which resulted in the promotion of photosynthesis and growth. Therefore, this light source can be advantageous for the accumulation of tomato plants soluble carbohydrates. The best effect observed on leaf fructose and glucose concentration was related to red light which was different to what Wang et al. (2009) had suggested. They found that contrary to white light, there was a significant reduction in the total carbohydrates, sucrose and starch contents in cucumber seedlings under Red light. This finding can be associated with plant species differences. In fact, sucrose cleavage or greater photosynthetic capacity might cause sucrose and starch increase and sustained elongation growth under red light (Ahmed et al., 2013).

Considering the effects of blue and red light spectrum on leaf vegetables, the aim of this study is to investigate and compare the effects of different blue and red lights combinations on biological, biochemical and photosynthetic characteristics of cress plant as an important leaf vegetable and medicinal plant with the control treatment. This study is conducted to introduce the most ideal growing condition of cress in terms of lighting ratios of blue and red spectrum combinations and to compare the use of this technology with the control treatment in which natural sunlight is used.

2. Materials and method

2.1. Plant materials and growing conditions

To examine the effects of blue and red lights, the present study was implemented and conducted as a pot experiment inside a greenhouse through a completely random plan with three lighting treatments including natural light (control), 60% red light +40% blue light, and 90% red light +10% blue light. The treatments were repeated 3 times at the research greenhouse of the faculty of agriculture, Ferdowsi University of Mashhad, with a latitude of 36° 16′ North and a longitude of 59° 36" East, altitude of 985m from sea level, mean temperature of 15–27 °*C* and relative humidity of 40–70%. As the temperature inside the greenhouse was recorded by receivers connected to the greenhouse central system, the ceiling windows and/or ventilators were automatically activated in case of temperature rise. Each lighting treatment consisted of 3 pots with 3 repetitions, amounting to a total of 27 pots, and 15 cress seeds were planted inside each pot. The mean data of each pot 35 days after sowing the seeds were examined via statistical analysis. In this study, plastic pots with a height of 40 cm and diameter of 30cm were used. The cultivation bed for the plants included a mixture of 40% peat moss, 40% coco peat, and 20% pearlite The irrigation system of the plants was that each pot was watered at amount of 50 cc per day until it reached a height of 10 cm and were subsequently fed with half Hoagland solution every two days. Measurements were carried out 35 days following the planting and completed growth of the plants.

2.2. Lighting treatments

Plants were illuminated by light emitting diodes (LEDs) with different percentages of red (R, 661 nm) and blue (B, 449 nm) lights. Three spectral treatments were used in this study, namely 90%R+10%B, 60% R+40%B and control. The photoperiod was 12/16h (day/night), and photosynthetic photon flux density (PPFD) was 168 ± 10 μmol m^{-2} s^{-1}. The LED lights were prototypes from General Electric Lighting Solutions (Salid, Karamax, Iran). These consisted of 0.26 m, 0.06 m, and 0.05 m linear fixtures, on which an array of 6 LEDs was placed. Moreover, irradiance was measured routinely using a quantum sensor (MQ-200; Apogee Instruments, Logan, UT). Also, photosynthetic photon flux density intensities and light spectra were monitored using a light meter (Sekonic C-7000, Japan). The relative spectra of the light treatments (percent of total PPF) are shown in Figure 1. It is to be mentioned that the

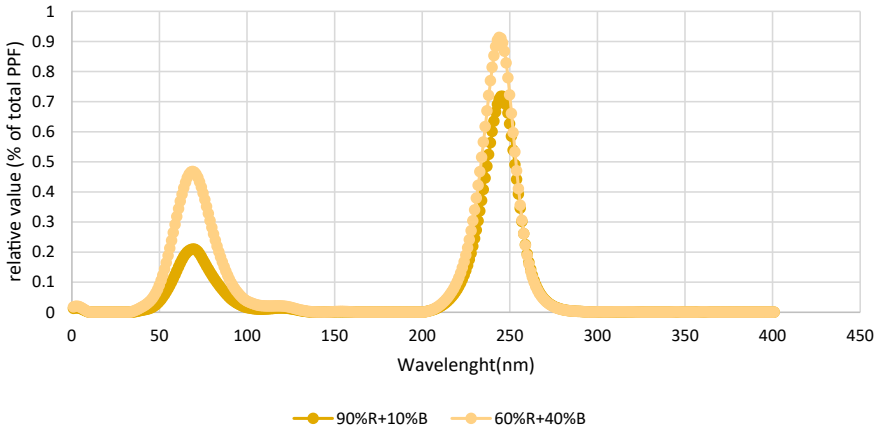

Figure 1. Relative spectral photon flux of the light sources red and blue (RB) utilized.

distance between lamps and plants were adjustable during different stages of the growth via metal clips. At the control experiment unit, natural sunlight was used. The plants growing environment was completely covered using special plastic covers in order to avoid light interference while the lamps were on.

2.3. Examined traits

2.3.1. Measurement of photosynthetic rate, Fv/Fm and Φ_{PSII}

Photosynthesis and chlorophyll fluorescence were measured in several stages in greenhouse conditions successive days (days 1, 3, 5, 7 after see two expanded leaves) to study the effects of leaf acclimation. We randomly selected three plants per treatment for these measurements. The third fully expanded leaf from the apex for Cress was labeled. On the first day, the daily chlorophyll fluorescence pattern was also recorded (every 2 h from 09:00 to 17:00). Leaf gas exchange was measured using the Li-6400 portable gas exchange system (LiCor Inc.US). The CO2 concentration entering the leaf chamber was adjusted to 400 μmol mol-1 supplied by a CO2 gas container, leaf temperature was maintained at 22 °C. After 1 month, photosynthesis and chlorophyll fluorescence were measured again, but this time on the youngest and fully developed leaf under greenhouse conditions with three replicate plants in order to study if there were remaining effects on the newly developed leaves. Chlorophyll a fluorescence was measured using a portable amplitude modulation fluorometer (PAM-2500cd, Walz-Germany). Next, the leaf was dark-adapted for 30 min, and a 0.6 s saturating light (3450 μmol m^{-2} s^{-1}) was given to obtain the maximal and minimal fluorescence yield (Fm and F0). Then, the leaf was light-adapted for 5 min with continuous actinic light at 600 μmol m^{-2} $s^{-1}$and saturating pulses every 25 s, and the maximum (Fm') and the steady-state fluorescence (Fs) signals were recorded. The actinic light was turned off and a far-red pulse was applied to obtain the minimal fluorescence after PSI excitation (F0′). Furthermore, the maximum photochemical efficiency of photosystem II (Fv/Fm) was calculated as Fv/Fm = (Fm-F0)/Fm; photosystem II operating efficiency (ΦPSII) was calculated as (Fm'- Fs)/Fm'; photochemical quenching (qP) was calculated as qP = (Fm'-Fs)/(Fm'-F0′); and non-photochemical quenching (NPQ) was calculated as NPQ = (Fm–Fm')/Fm' (Baker, 2008).

2.3.2. Stomatal conductance, transpiration and chlorophyll index

Stomatal conductance and transpiration were measured by AP4 POROMETER (Delta-T UK) apparatus and leaf chlorophyll index by Chlorophyll Meter, SPAD-502 (Konica, Minolta,Tokyo) model in the third leaf of the plant.

2.3.3. Total amount of soluble carbohydrates

For this purpose, 0.5 g of frozen leaf sample was crushed with 5 ml of 95% ethanol in porcelain mortar. The supernatant was collected from the top of the extract and the extraction process was continued in a two-step rinse, each of which was performed with 5 ml of 70% ethanol on the remaining sediments of the extracts.The collected alcoholic extracts were centrifuged for 10 min with the speed of 3500 rpm at 4 °C, and the method of Irigoyen et al. (1992) was used to measure total soluble sugars.

2.3.4. The amount of leaf starch

In order to measure starch, Marshall (1986) method was used. To this end, 5 ml of 1.1% acid chloride was added to the residues of the collected alcoholic extract from the soluble carbohydrate part of which all the alcohols (5ml) were vaporized. The residues were then placed in a hot water bath at 100 °C for 30 min. Next, 10 ml of distilled water was added to the samples. Then, 1 ml of the extract was poured into a 10 ml Falcon and it was frozen at 0 °C in ice. Then 5 ml of Anthrone Reagent was added to each sample. The samples were again placed in a hot water bath at 100 °C for 11 min, and they were then rapidly lowered to 0 °C in ice. Finally, they were read by a spectrophotometer at the wavelength of 630 nm.

2.4. Statistical analysis

There were 15 cress shrubs at each pot; the mean data of each pot during the growth period was examined in statistical analysis. The data were subjected to two-way analysis of variance (ANOVA) and the LSD test was used as a post-test. $P \leq 0.01$ was considered not significant. Charts were drawn using Excel 2019 software.

3. Results

3.1. Effects of light treatments on photosynthetic rate (P_G), Fv/Fm[1] and ΦPSII[2]

What the data show, is a positive and significant ($p \leq 0.01$) effect of using LED lamps as artificial light on photosynthetic rate (PG), Fv/Fm and ΦPSII characteristics. The highest amounts of each of these characteristics in 60R: 40B treatment were 12.4, 0.87 and 0.92 (μmol CO_2 m^{-2} s^{-1}), respectively. Based on these data, it was found that adding more blue light improved the photosynthetic factors compared to the other treatments (Figures 2, 3, and 4).P_G levels were also 38/83% decreased in cress leaves grown in natural sunlight compared to the 90R:10B treatment (Figure 2). Under the 90R: 10B light treatment, Fv/Fm and ΦPSII amounts were found to be 0.66 and 0.81 in the cress leaves, respectively. In the study of these characteristics, the lowest amount was observed in the control light treatment.

[1] Maximum photochemical efficiency of photosystem II.
[2] Photosystem II operating efficiency.

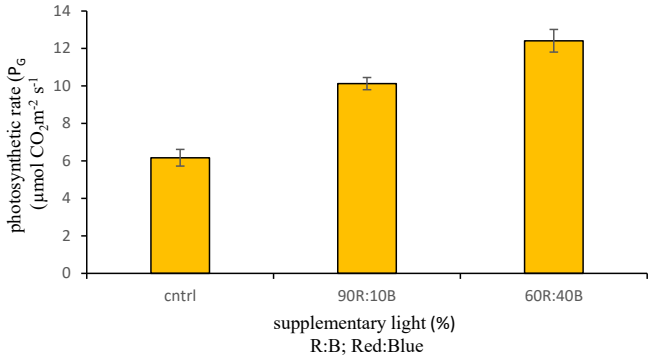

Figure 2. Comparison of the Effect of Optical Spectra on photosynthetic rate (P_G) of the Crop Plant ($p \leq 0.01$) Different letters indicate significant differences between treatments by LSD test.

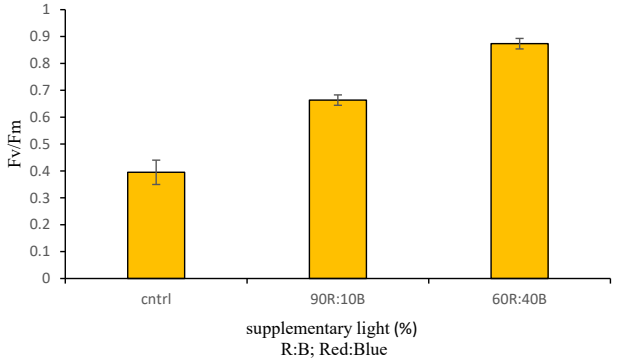

Figure 3. Comparison of the Effect of Optical Spectra on Fv/Fm of the Crop Plant ($p \leq 0.01$) Different letters indicate significant differences between treatments by LSD test.

3.2. The effects of light treatments on stomatal conductance

Based on the results of data analysis of variance, stomatal conductance of cress at probability level of 1% was affected by different light treatments. The recorded stomatal conductance increased with increasing the red light ($p \leq 0.01$), so that in the 90R: 10B treatment the maximum stomatal conductance was observed to be 0.3 ($cm.s^{-2}$). As shown in Figure 5, the decreasing trend ($p \leq 0.01$) in the rate of stomatal conductance due to the decrease in red light was visible. This amount was 0.27 ($cm.s^{-2}$) in the 60R: 40B treatment. Also, in the control treatment, the light source of which was natural sunlight, the lowest amount of 0.11 was observed.

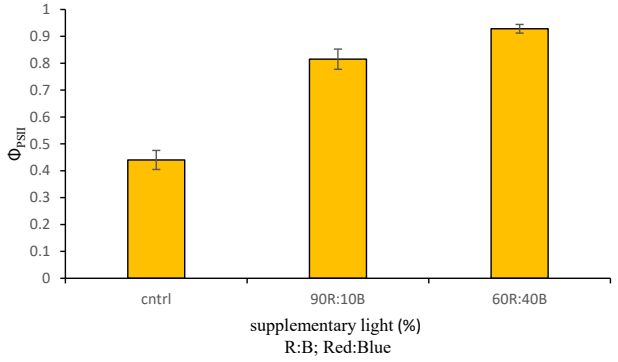

Figure 4. Comparison of the Effect of Optical Spectra on Φ_{PSII} of the Crop Plant ($p \leq 0.01$) Different letters indicate significant differences between treatments by LSD test.

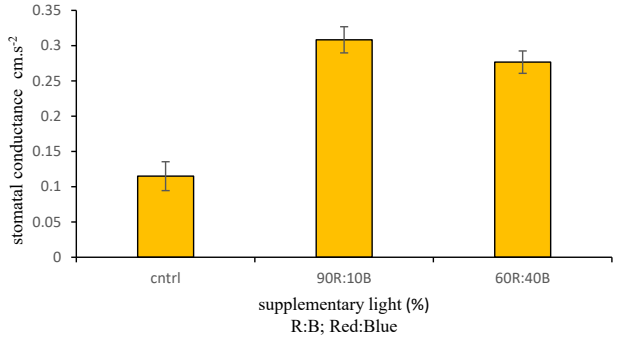

Figure 5. Comparison of the Effect of Optical Spectra on the Stomatal conductance of the Crop Plant ($p \leq 0.01$) Different letters indicate significant differences between treatments by LSD test.

3.3. The effects of light treatments on transpiration

Significant increase ($p \leq 0.01$) in transpiration rate of cress was observed when using LED lamps with different light percentages including 60R: 40B and 90R: 10B compared to the control treatment (sunlight) (Figure 6). The highest transpiration rate was observed in the treatment that had higher percentage of blue light than the other treatments (60R: 40B). According to data analysis, this amount was 25/83 ($mol.m^{-2}.\ s^{-1}$), which was significantly different from the other treatments ($p \leq 0.01$). Moreover, the transpiration rate in plants treated with 90R:10B light treatment was 18.83 ($mol.m^{-2}.\ s^{-1}$), which was significantly higher, compared to the control (sunlight) treatment ($p \leq 0.01$); on the other hand, the lowest transpiration rate in control (sunlight) treatment was 5.5 ($mol.m^{-2}.\ s^{-1}$).

3.4. The effects of light treatments on the amount of total soluble carbohydrates

The evaluation of different light treatments showed that the total carbohydrate content in the cress plant was affected by LED lights at 1% probability level, and a significant increase in total carbohydrate content was observed in comparison with the plants grown under natural sunlight conditions; in such a way that with the use of 1% LSD, plant carbohydrate content increased by 36% in 90R: 10B treatment compared to natural light treatment (Figure 7). It should be noted that the 60R: 40B treatment contained 4.54 ($mg.\ g^{-1}$ FW) carbohydrates.

3.5. The effects of light treatments on the amount of leaf starch

The starch content of the leaves was significantly increased by the use of LED lamps during the growth period ($p \leq 0.01$) (Figure 8). Both of the

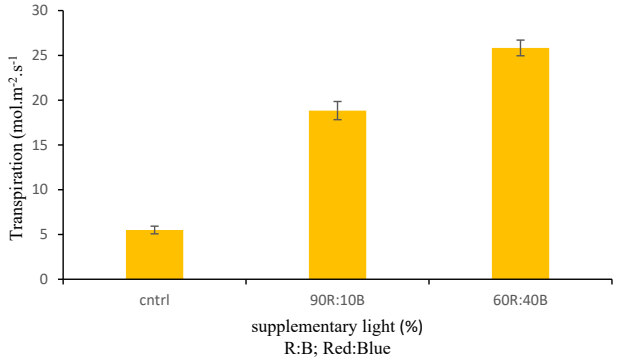

Figure 6. Comparison of the Effect of Optical Spectra on the Transpiration of the Crop Plant ($p \leq 0.01$) Different letters indicate significant differences between treatments by LSD test.

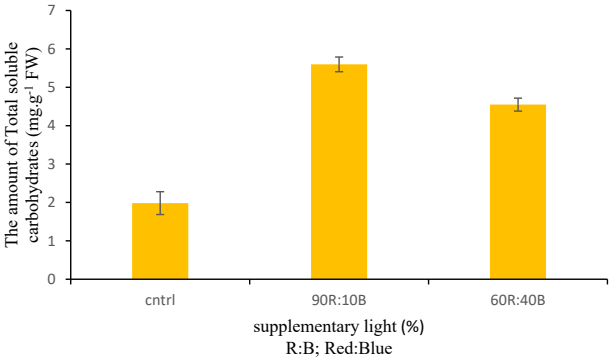

Figure 7. Comparison of the Effect of Optical Spectra on the amount of Total soluble carbohydrates of the Crop Plant ($p \leq 0.01$) Different letters indicate significant differences between treatments by LSD test.

different percentages used in this experiment, including 60R: 40B and 90R: 10B, could increase the amount of cress leaves starch up to 83.12% in comparison with the control treatment, which only received sunlight. It should be mentioned that the lowest starch content was observed in the leaves of the control treatment which was 1.09 (mg.g $^{-1}$ F).

3.6. The effects of light treatments on leaf chlorophyll contents (SPAD)

The application of artificial light during planting period had a significant effect on chlorophyll content of cress leaves with 1% LSD. According to the results shown in Figure 9, the higher the percentage of blue light, the significantly higher the chlorophyll content compared to the other treatments. As an example, the highest chlorophyll content (48.18) was observed in the 60R: 40B treatment, which received the highest percentage of blue light. Significant differences were observed between the light treatments. The 90R: 10B treatment had the amount of 33.45 of chlorophyll content, and the lowest amount that was 25.5, was observed in the control treatment.

4. Discussion

4.1. Photosynthetic rate (P_G), Fv/Fm and ΦPSII

As shown in the results of this study, the treatment that had a higher blue light spectrum (60R: 40B) than the other light treatment improved the photosynthetic condition in the cress. In general, the use of light treatments had better effects than the control treatment. Light is a prominent source of energy for photosynthesis and it is essential for plant growth and development. Plants are also known as creatures that can respond to the intensity, quality or the color of ligh (Neff et al., 2000).

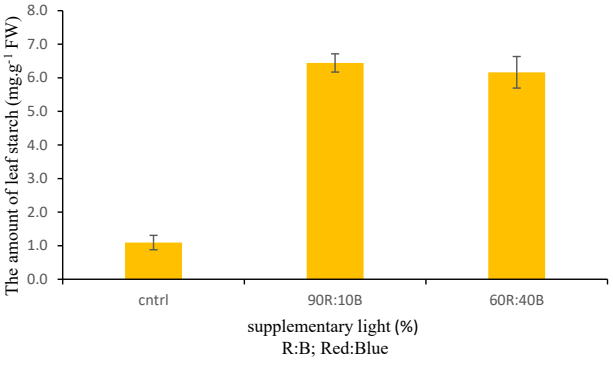

Figure 8. Comparison of the Effect of Optical Spectra on the amount of leaf starch of the Crop Plant ($p \leq 0.01$) Different letters indicate significant differences between treatments by LSD test.

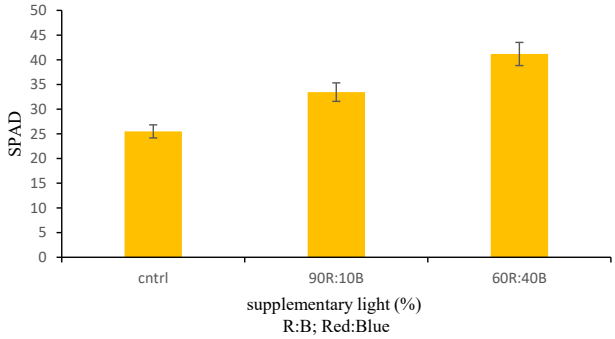

Figure 9. Comparison of the Effect of Optical Spectra on SPAD of the Crop Plant ($p \leq 0.01$) Different letters indicate significant differences between treatments by LSD test.

However, how light quality can affect plant growth and development, particularly photosynthesis, remains unclear. A few studies have been conducted to examine light quality effects on plants by using blue and red colors (Hogewoning et al., 2007). These studies illustrated that "under blue light, plants had a greater stomatal opening, higher Chl a/b ratios, smaller amounts of light-harvesting Chl a/b-binding protein in photosystem II (PSII), higher photosynthetic electron-transport activity per unit of Chl content, and higher Rubisco activity than plants grown under red light" (Eskins et al., 1991; Sharkey and Raschke, 1981). Recently, Matsuda et al. (2004) found that rice plants grown under the combination of red and blue lights had higher photosynthetic rates in their leaves compared to those grown under red light alone (Ma et al., 2001). Therefore, how light quality can affect both photosynthesis and plant growth requires further research by using more light colors. Our results demonstrate that plants grown under red and blue lights show an increase both in growth and photosynthetic rate compared to the ones grown under sunlight.Having different Chl a/b ratio and photosynthetic rate, the leaves grown under sunlight or other lights demonstrate differences in photosynthetic rate and pigment composition (Sarijeva et al., 2007; Zhang et al., 2016). Such modifications allow adapting photosynthetic efficiency to light spectral quality variations. Blue photons are also reported to be capable of increasing the Chl a/b ratio (Abidi et al., 2013), which is in accordance with the decrease in the size of the PSII light-harvesting antenna complex (Bailey et al., 2001). Additionally, as photosystem I (PSI) absorbs red light preferentially, its stoichiometry can change due to long term exposure to red and blue lights, in terms of a compensatory increase in photosystem II to maintain a balanced excitation rate of both photosystems (Shevchenko et al., 1996). However, it has been suggested that the electron transport is inhibited by monochromatic red light irradiation from photosystem II donor side to photosystem I (Miao et al., 2016); as a result, it causes an imbalance of light energy distribution for the photosystems (Tennessen et al., 1994), which leads to the photosynthetic performance inhibition. According to Terfa et al. (2013), higher blue ratios can positively affect the photosynthetic apparatus development in Rosa hybrid. Furthermore, Shengxin et al. (2016) reported that "rapeseed leaves grown under pure blue or a high blue photon ratio showed higher ability to utilize high photon fluxes". In addition, the use of LED light can result in the highest photosynthetic rate as its blue and red radiations can be effective for photosynthesis (Savvides et al., 2012), so we can saw a similar result in this experiment, blue and red light spectra in combination were effective for photosynthesis in the cress plant. "The photosynthetic rate at 25 days after sowing was the highest in the LED light and the lowest in the fluorescent light and red light" (Han et al., 2019). It should be taken into consideration that Fv/Fm and photosynthesis are sensitive to light stress, and Fv/Fm can be decreased by red light according to Baker (2008). For instance, a study conducted by Zheng et al. (2020) showed that acclimation stress was distinguished by the diurnal pattern for the leaves of two ornamental plants grown under red light; yet, no response difference was detected for

the other light quality pretreatments. In spite of the fact that negative effects of red light in Chrysanthemum were not crystal clear, ΦPSII was reduced under this light in comparison with the other qualities in *Spathiphyllum*. Based on the study of Trouwborst et al. (2016), the imbalances in light energy distribution between PSII and PSI photosystems cannot be identified as the source of red light negative effects since leaves were under natural light; however, they can be attributed to the adverse effects on leaf structure and thylakoid development under red light because as Savvides et al. (2012), monochromatic red light is able to damage the photosynthetic machinery.

4.2. Stomatal conductance and transpiration

According to the results shown in Figure 5, with a slight difference, both light treatments had the highest stomatal conductance in the cress plant; nevertheless, the stomatal conductance of the plants grown in natural light was at its lowest. Stomata are like holes that are located in the leaf epidermis and are used for gas exchange between the plant and the atmosphere. Some researchers have emphasized the importance of blue light in the opening of stomata (Kraepiel and Miginiac, 1997). A study on Xanthium Strumarium L showed that blue light stimulates stomatal conductance 10 to 20 times more than red light (Sharkey and Raschke, 1981). Based on these studies, blue light is the only light that influences the stomatal conductance at low light intensity, which is in harmony with the results of this study. Based on recent findings in the study of the effects of blue and red spectra on plant leaf stomata, the data showed that blue light has an effect on the opening and closing of stomata through direct effects on guard cells as well as indirect effects on other leaf cells (Ballard et al., 2019).There are also studies with contradictory results; for example, in a study on radish, spinach, and lettuce plants, it has been demonstrated that the stomatal conductance of plants growing under fluorescent light (with little or no blue light) was higher than the plants grown under red LEDs (Yorio et al., 2001).The almost immediate effect of light quality on stomatal aperture is notable: red and blue lights stimulate stomatal opening leading to stomatal conductance (gs) with the help of different mechanisms affecting guard cells' turgor pressure (Lawson, 2009; Zeiger et al., 2002). It has to be noted that light quality long term effects can also be as remarkable as gs, having effects on stomata size, number and distribution over the upper and the lower surfaces of leaves (Franks and Beerling, 2009). Studies done by (Scoffoni et al., 2008) illustrate that higher light intensity and duration can induce a greater leaf; whereas, light quality can influence leaf, as short-term exposure (not during growth) of the sun- and shade leaves to different light qualities resulted in different values of the leaf (Sellin et al., 2011). (Karlsson, 1986) have shown that blue and red lights can stimulate stomatal opening more than other light wavelengths which can contribute to an increase in the dry matter production. Furthermore, Goins et al. (1997) examined wheat plants developed under red LED light supplemented with blue light, and observed higher photosynthetic rates as well as stomatal conductance increase in the plants' leaves.In fact, it can be concluded that the effect of light quality varies with plant species, their growth stage and environmental conditions. The highest transpiration rate in the cress plant was observed in the leaves with the highest percentage of red light in their light composition; nonetheless, compared to the control light treatment, both blue and red treatments had the highest transpiration rates with different percentages. The transpiration rate at the wavelength of red light decreased compared to the other light treatments, which is consistent with the results of Lee et al. (2007). The combination of red and blue lights has an additive effect on transpiration, which can be attributed to stopping (interruption of) the differentiation of stomata under red light (Lee et al., 2007). The reason for the interruption of stomata differentiation under red light can be explained by the lack of cryptochromes and phototropins that work in conjunction with blue light to cause stomatal development and opening (Kang et al., 2009).Several environmental variables such as lighting conditions and atmospheric CO_2 concentrations can affect the number of stomata developed in epidermis. As a matter of fact, although the understanding of the interacting signals network that regulates stomatal differentiation, has improved (Le et al., 2014), the effects of the abovementioned environmental factors on stomatal patterning is still unclear. Blue-light photoreceptors, cryptochromes (CRYs), red/far-red light photoreceptors, and phytochromes (Phy) are considered as important regulating photoreceptors (C. Lin, 2002). Stomatal development is controlled by both red and blue lights, the independent regulatory pathways of which are also clear. "Blue light suppresses the expression of CRYs and directly regulates stomatal formation by controlling constitutive photomorphogenic 1(COP1) (C. Lin, 2002). On the contrary, the regulatory function of red light is detected by photoreceptor phytochrome B (phyB), which regulates the cell fate changes during stomatal development while being active within both the stomatal lineage and nonepidermal tissues.

4.3. The amount of total soluble carbohydrates, leaf starch and leaf chlorophyll contents (SPAD)

Light quality also regulates plant carbohydrate metabolism. In cress, based on the results observed, light treatments increased the amount of carbohydrates, which was a significant increase compared to the control treatment. Blue light effectively accelerates carbohydrate accumulation in storage tissues and is considered as a regulator of photosynthesis in plants (Fan et al., 2013). In blue light, protein increases and carbohydrate decreases. This increase can be due to the activation of protein biosynthesis in this light, which results in the reduction of carbohydrate content because of carbohydrate degradation through assisting the synthesis of amino acids and proteins. Based on the findings of other researchers that were consistent with our results, it can be said that the treatment with the bluest light had the highest amount of carbohydrates. It has also been reported that under photosynthetic conditions, blue light converts carbon dioxide into amino acids and organic acids, and red light enhances the stabilization of starch and sucrose. On the other hand, it has been recognized that blue light is able to activate and promote new syntheses of pyruvate kinase; the high levels of which result in the formation of more organic acids and thus an increase in the synthesis of amino acids (Barro et al., 1989). Moreover, increased levels of carbohydrate under red light have also been demonstrated on cabbage, soybean, and rapeseed (Lin et al., 2013; Wang et al., 2009). As a matter of fact, red light is required for the development of photosynthetic apparatus and starch accumulation, while blue light is useful for chlorophyll formation, chloroplast development, and stomatal opening (Heo et al., 2002; Wu et al., 2007), in rcress, based on the observed results, the presence of red light treatment increased the accumulation of starch, which was a significant increase compared to the control treatment. As we know, stomatal movements are stimulated by the changes in the osmoregulation of the guard cells via blue light. At this time, blue light activates an H-ATPase in the plasma membrane of the guard cell, which leads to stimulating the starch degradation, the biosynthesis of malate, the accumulation of soluble material inside the guard cells, and eventually the stomatal opening (Taiz et al., 2015). The findings of Shin et al. (2008) are also in line with our study. They have stated that in a type of orchid flower, simultaneous red and blue light irradiation produces more starch than individually irradiated red and blue lights as well as fluorescent light. Moreover, it was observed that the treatment with the higher percentage of red light had more starch in its leaves. The quality of the light spectrum can influence the composition of the pigments and can directly affect the quantum yield of the plant for carbon dioxide fixation. For example, it has been reported that blue light stimulates flavonoid synthesis, and it can be seen that blue light (400–500 nm) which is abundant in the PAR (400–700 nm) spectrum, stimulates the side pigments (Hogewoning et al., 2010). Matsuda et al. (2016) noted that blue light is involved in the light adaptation on spinach chloroplast surface. Extensive studies have been done on the importance of blue light in artificial light conditions by the use of LEDs that show how blue light affects chlorophyll content in wheat, potato, Arabidopsis and spinach

plants. These results were in line with our findings on cress leaves which show the significant effect of blue light on chlorophyll content as the treatment with a higher blue light percentage had a higher chlorophyll content.According to Lichtenthaler (1987), blue light is believed to be related to the 'sun-type' characteristics such as high photosynthetic capacity at the chloroplast level, also according to report Ajdanian et al. (2019) the more the percentage of the blue light, the more the amount of chlorophyll was considerably increased. The highest amount of chlorophyll a,b.T was observed under 60R:40B treatment with values of 9.4, 5.68, and 15.09 mg g^{-1} FW leaf, respectively. Most part of the research conducted to assess blue light effects on the leaf or whole-plant levels such as Matsuda et al. (2008), have compared broad-band light source responses with blue-deficient light responses or plants grown under blue or a combination of red and blue lights with plants grown under red light alone (Matsuda et al., 2004).

5. Conclusion

Growth and development of plants under natural conditions are affected by numerous genetic and environmental factors that alter the growth and development of the plant in the absence of genetic constraints and environmental factors. One of these significant environmental factors affecting plant growth is light. Leaf vegetables are of great importance as they are primarily considered a food source for humans; thus, the quality of their production is also incredibly remarkable. In this experiment, the leaf vegetable of cress was used as an important food source to investigate the amount of photosynthetic factors, carbohydrate, and starch using artificial light (blue and red LEDs). The blue and red light spectra had positive effects, in a way that the 60R: 40B light treatment which had a higher percentage of blue light compared to the control light treatment that used natural sunlight, experienced an increase of 50% in photosynthetic factors (PG, Fv/Fm and ΦPSII) because blue light has a greater effect on the growth and development of chlorophyll and chloroplast; therefore, the greener and more vibrant the leaf, the higher its quality for consumption. The light treatments had also better effects than the control treatment; as a result, it can be said that both wavelengths (blue and red) are essential for the better and full growth of the plant. Therefore, it can be suggested that it is possible to use these lamps for better economic production under controlled conditions (greenhouses). The exact ratio between the blue and red light spectra varies according to the type of plant and the light needs of the plant. But what is important is that the presence of blue light along with red light is necessary for better plant growth, even if we use a small amount of blue light.

Declarations

Author contribution statement

Ladan Ajdanian: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Mehdi Babaei: Analyzed and interpreted the data; Wrote the paper.

Hossein Aroiee: Conceived and designed the experiments; Analyzed and interpreted the data.

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Data included in article/supplementary material/referenced in article.

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The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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