CO₂ enrichment and increasing light intensity
till a threshold level, enhance growth and water
use efficiency of lettuce plants in controlled environment

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Abstract

Carbon dioxide (CO₂) and light intensity are the two main environmental drivers known to play important roles in crop growth and yield. In the current study, lettuce seedlings were exposed to four different light intensities [(75, 150, 300 and 600 Photosynthetic Photon Flux Density (PPFD))] and four different concentrations of CO₂ (400, 800, 1200 and 1600 ppm). By increasing light intensity and CO₂ concentration growth parameters such as fresh weight, dry weight and leaf area were stepwise increased from 75 to 300 PPFD and from 400 ppm to 1200 ppm CO₂ concentration. Maximum fresh weight was observed in 300 PPFD under both 1200 ppm and 1600 ppm CO₂ concentrations. Highest dry weight was obtained in plants exposed to 300 and 600 PPFD under both 1200 and 1600 ppm CO₂ concentrations. Highest leaf area was detected in 300 PPFD under both 1200 and 1600 ppm CO₂ concentrations. Widest stomatal pore aperture was detected in 600 PPFD under 400 ppm and 800 ppm CO₂ concentrations. Evapotranspiration increased in a light intensity and CO₂ concentration-dependent manner; higher light intensity or higher CO₂ concentration, more evapotranspiration. Highest water use efficiency (WUE) was achieved in plants exposed to 300 PPFD under 1200 ppm CO₂ concentration. In conclusion, to achieve best growth performance and WUE, lettuce should be produced under 300 PPFD light intensity and 1200 ppm CO₂.

Keywords: carbon dioxide; lettuce; light intensity; stomatal properties; WUE
Introduction

Greenhouse industry is vastly expanding since it is an environment with high capacity of controlling environmental factors. Production of plants in greenhouses leads to improve in crop yield, prolonged production period, better product quality, and efficient use of chemicals (van Straten et al., 2010). Nowadays, there is an increasing tendency to produce leafy vegetables in protected systems and plant factories (Kozai et al., 2015). Plant growth, development and water requirement depend on many environmental parameters such as humidity, temperature, CO$_2$, and light (Brengrup et al., 2001). Biomass production in plants directly depends on photosynthesis. Light and CO$_2$ are the two environmental cues that directly influence photosynthesis. Among environmental factors, light is the main and primary source of energy for plants (Aliniaefard et al., 2018). Light has different attributes that affect plant growth, development, and water relations (Quail, 2002; Yu et al., 2016; Aliniaefard et al., 2018). Different properties of light including intensity, quality (spectrum) (Zou et al., 2019) and duration (photoperiod) (Sørensen et al., 2018) influence many aspects of plant growth and development (Bayat et al., 2018). One aspect of light that is important for growing plants is its intensity, which attracted so much attention for plant production, especially in closed production systems (Centritto et al., 2000; Kozai et al., 2015). To fit the fluctuations in lighting environment, many adaptations are evolved to decrease/increase incident light to the leaf surface. These adaptations occur in morphological, physiological or biochemical levels (Zhang et al., 2003; Zhao et al., 2017; Aliniaefard and van Meeteren, 2018a). Increase or decrease in optimal light intensity can negatively influence photosynthesis and plant performance (Bowes et al., 1972; Al-Khatib and Paulsen, 1989). Low light intensities lead to stem and leaf elongations and other morphological modifications to maximize absorption of the available light, meeting the demand for photosynthesis (Steinger et al., 2003), while high light intensities cause plant compactness and reduction in leaf area expansion to decrease energy absorption in response to elevated irradiance (Givnish et al., 2004; Morais et al., 2004; Thomas et al., 2004; Matos et al., 2009). Since light has a direct effect on stomatal opening and as a result on transpiration and photosynthesis, therefore it is one of the main drivers of WUE of the plants (Giday et al., 2014; van Meeteren and Aliniaefard, 2016; Lanoue et al., 2017; Aliniaefard and van Meeteren, 2018b).

CO$_2$ is an essential input for crop photosynthesis. Elevated CO$_2$ causes significant impact on crop growth and production in greenhouses (Fanourakis et al., 2011; Li-quan et al., 2016). It has been reported that increase in CO$_2$ concentration leads to numerous physiological and morphological changes in plants, in particular changes in photosynthesis, nutrient uptake and translocation, stomatal properties, gene expression and enzyme activity and consequently water use efficiency have been reported (Ainsworth et al., 2007; Damatta et al., 2010; Tausz et al., 2013; Vahdati et al., 2017). These processes are expected to improve crop growth, water status, water requirement and finally WUE of the plants. CO$_2$ enrichment may affect plant’s transpiration through changing stomatal properties (Lemon, 1983; Idso et al., 1988; Vahdati et al., 2017). Former investigation on CO$_2$ elevation in greenhouses and other closed production systems suggested that CO$_2$ elevation promote photosynthesis and alters stomatal morphology and behavior (Long et al., 2004; Long et al., 2006; Vahdati et al., 2017). Elevation in CO$_2$ promotes plants growth, and crops yield by increase in net photosynthetic rate and inhibition of photorespiration in C$_3$ plants (Jitla et al., 1997; Long et al., 2004; Rogers et al., 2004; Wu et al., 2004).

It has been reported that stomatal aperture widens by increase in light intensity, while it decreases as a result of exposure to elevated CO$_2$ concentration; both control transpiration in the plants (Franks and Beerling, 2009) and consequently WUE. Long-term effect of both elevated CO$_2$ concentrations and different light intensities on growth and WUE has been extensively studied. However, detailed investigation on the effects of these two main drivers of photosynthesis on stomatal properties and WUE are scarce. Therefore, the objectives of the present study were to: I) investigate the effect of different light intensities and CO$_2$ enrichments on growth and stomatal properties during lettuce growth under controlled conditions, II) Evaluation of impact of different light intensities and CO$_2$ enrichments on evapotranspiration and WUE of
lettuce and III) Find the best light intensity and concentration of CO₂ for culture of lettuce in closed production system.

Materials and Methods

Plant material and growth condition

Lettuce (Lactuca sativa cv. ‘Partavousi’) seeds were germinated in trays filled with a mixture of cocopeat and perlite (3:1, V:V) in a growth chamber under 250 photosynthetic photon flux density (PPFD; determined using Sekonic C7000, Japan). When two true leaves were emerged, the seedlings were transplanted into plastic pots with 15 cm diameter and 20 cm depth containing cocopeat; then placed in environment-controlled growth chambers. The plastic pots were filled with soil and placed in 16 growth chambers (l × w × h = 1 m × 1 m × 1 m) with combination of red and blue LEDs with peaks at 600 nm to 685 nm for red and 415 nm to 500 nm for blue LEDs. LED light panels were used to make different light intensities inside the growth chambers and also to restrict production of heat by the light sources. Same combination of red and blue LEDs (3:1) was used in all 16 growth chambers, since based on the previous reports they are the main light spectra for photosynthesis and growth of lettuce plants (Pennisi et al., 2019). The lighting period was 12/12 h light/darkness. All of the growth chambers had a temperature of 25±2 °C, and a relative air humidity of 50±5%. Ambient CO₂ was 400 ppm (determined by Trotec, BZ30, Germany), for adjusting higher concentrations, CO₂ was injected by CO₂ gas capsules into the growth chambers. The CO₂ concentration was controlled by solenoid valves, timer and air CO₂ sensor and it was recorded by air CO₂ sensor data logger (Trotec, BZ30, Germany) every 2 seconds.

Plants were irrigated with half-strength of Hoagland solution for the first 10 days after transplantation, thereafter irrigation was done with modified full Hoagland solution (Table 1).

| Ingredients       | Concentration (mg L⁻¹) for Stoke 1 Molar | Volume for 1 L |
|-------------------|-----------------------------------------|----------------|
| KH₂PO₄            | 136.1                                   | 1 ml           |
| KNO₃              | 101.1                                   | 5 ml           |
| Ca(NO₃)₂.4H₂O     | 236.1                                   | 5 ml           |
| MgSO₄.7H₂O        | 246.5                                   | 2 ml           |
| H₃BO₃            | 2.86                                    | 1 ml           |
| MnCl₂.4H₂O        | 1.81                                    | 1 ml           |
| CUSO₄.5H₂O       | 0.051                                   | 1 ml           |
| H₃MoO₄.4H₂O      | 0.09                                    | 1 ml           |
| Na₃MoO₄.2H₂O     | 0.12                                    | 1 ml           |
| ZnSO₄.5H₂O       | 0.22                                    | 1 ml           |
| FeEDDHA           | 4.04                                    | 0.01 ml        |

Experimental treatments under different light intensities and CO₂ enrichment

This experiment was carried out based on a factorial experiment. It has been shown that light intensity more than 600 PPFD induces serious stress on lettuce plants (Fu et al., 2012) therefore, four different light intensities including 75, 150, 300 and 600 PPFD (determined using Sekonic C7000, Japan) were used in the growth chambers. Four different levels of CO₂ including 400, 800, 1200 and 1600 ppm were also used (CO₂ sensor, Trotec, BZ30, Germany) (Figure 1). Generally, there were 16 different treatments and in each growth chamber, twelve pots were placed under each treatment, in total 192 pots were used for this experiment.

For subsequent irrigations, pots were used as a micro-lysimeter. In this method, irrigation water rate was calculated by difference in the weight of pot in two times of irrigation which was the amount of evapotranspiration from every pot.
Measurements and calculations

For growth analysis, leaves of three plants per each treatment were detached every 10 days and their fresh and dry weights were recorded. For measuring leaf area, the leaves were scanned by a scanner (HP Scanjet G4010) to obtain leaf surface area using Digimizer software (Digimizer V 4.1.1.0). Eventually, they were dried at 72 °C for 72 h until a constant mass was reached and weighted. To calculate specific leaf area (SLA), after measuring leaf area and leaf dry weight, it was calculated using the following equation (Aliniaeifard et al., 2016):

\[
SLA = \frac{\text{leaf area}}{\text{leaf dry weight}}
\]

To determine irrigation water use efficiency (WUE), the amount of water used during growth period was recorded. Under control condition, the relationship between the soil water evaporation and plant transpiration is inseparable (Ma et al., 2013). The water in the soil is the source of soil water evaporation. To measure the evaporation from the soil, in the absence of the plants soil evaporation was measured with the weighing method for all the pots. The lower limit for the soil was found to be 32% and the upper limit was 48% which was calculated in the lab by pressure plate instrument. The soil was irrigated when soil water content was below the lower limit. The WUE was calculated by using the following equation (Karam et al., 2007):

\[
WUE = \frac{\text{Dry weight}}{\text{Consumed water}}
\]

For investigation of stomatal morphological traits (stomatal length, stomatal width, and pore width), young fully developed leaves were used for stomatal measurement according to Aliniaeifard and van Meeteren (2016). Sampling was done in the middle of the leaf in the area between the tip and the base and away from the edges. For preparing the samples, the lower epidermis was coated by a thin layer of nail polish. After 5 min when the polish was dried a strip of transparent sticky type was used to take the dried polished for microscopic analysis. Image were taken by Omax topview software version 3.5 and further analyzed by using ImageJ software (U.S. National Institutes of Health, Bethesda, MD; http://imagej.nih.gov/ij/) to measure stomatal length, stomatal width, and pore aperture. For this measurement 100 stomata were analyzed for each treatment.

Statistical analysis

The data were evaluated by SAS software for windows (version 9.3) to determine significant differences. For analyzing growth parameters, data obtained from measurements of three plants for each treatment (per each time of harvesting) were used, and Duncan multiple comparisons test was used to compare the means.
Results

Increasing light intensity and CO₂ enrichment promote growth in lettuce

Light intensity and CO₂ levels significantly influenced lettuce growth parameters (Table 2). The result showed 20 days after onset of the experiment, plants exposed to 300 PPFD light and 1200 ppm CO₂ as well as those exposed to 600 PPFD light and 1200 ppm CO₂ had the highest fresh weight (Figure 2C), while plants exposed to 75 PPFD light under all CO₂ concentrations exhibited the lowest fresh weight (Figure 2A). At the day 30 all those plants exposed to 300 (except for 400 ppm CO₂) and 600 PPFD light intensities had higher fresh weight than fresh weight of those exposed to 75 and 150 PPFD light intensities. At the day 40, plants exposed to higher light intensities and CO₂ concentrations had better growth performance than those exposed to lower light and CO₂ levels (Figure 4). The fresh weight of lettuce increased by an increase in light intensity from 75 PPFD to 300 PPFD under all CO₂ concentrations; however, exposure to 600 PPFD light intensity had a negative influence on the fresh weight in comparison with the fresh weight of lettuce under 300 PPFD (Figure 4A). Under 600 PPFD light intensity, fresh weight increased in a CO₂ concentration-dependent manner. The highest fresh weight was detected in 300 PPFD light intensity and 1200 ppm concentration of CO₂ (Figure 4A), this increase was 54.74% more than the fresh weight of plants under standard condition (300 PPFD light intensity and 400 ppm CO₂). Lowest fresh weight was obtained from treatment with the lowest light intensity and CO₂ concentration, 75 PPFD light intensity and 400 ppm CO₂ concentration (Figure 4A).

For the dry weight, plants exposed to 600 PPFD light intensity and 1600 ppm concentration of CO₂ had considerably higher dry weight in whole through the growth period before the final harvest (10, 20 and 30 days after start of the treatments) when compared to dry weight of plants exposed to other light or CO₂ levels (Figure 3D). The lowest dry weight belonged to the plants exposed to 75 PPFD light intensity irrespective of CO₂ concentrations (Figure 3A). At the last harvest, similar results were obtained for the lettuce dry weight as those obtained for the fresh weight. Plants grown under 75 PPFD light intensity and 400 ppm CO₂ concentration had the minimum dry weight among plants grown under other light intensities and CO₂ concentrations, which was 79% decrease compared to the control treatment (Table 3). In conclusion, the effect of light intensity and CO₂ enrichment on dry weight was positive and there was a considerable increase in dry weight with the best result at 300 PPFD light intensity and 1200 ppm CO₂ (Table 3).

Table 2. Analysis of variance (P values) for assessed parameters for lettuce grown under different light intensities and CO₂ concentrations

| Independent variables | Degree of freedom | Mean squares | Fresh weight | Dry weight | Leaf area | Leaf area index | Evapotranspiration | WUE | Stomatal length | Stomatal width | Pore length | Pore width | Stomatal density |
|-----------------------|------------------|--------------|--------------|-----------|---------|----------------|-------------------|-----|----------------|-------------|-------------|-------------|------------------|
| Light intensity       | 3                | 14340.3      | 884538.3     | 524720.1  | 19881.9| 15.72         | 10.37            | 0.38| 2.38          | 6.25        | 10125.43    |
| CO₂                   | 3                | 1938.83      | 41850.78     | 15027.0  | 76**   | 3.72          | 57.61            | 21.94| 22.67        | 3245.41    |
| Light intensity * CO₂ | 9                | 312.03*      | 442539.70    | 88173.87 | 2.40***| 14.08**       | 5.50             | 2.82| 1.27          | 4205.09    |
| Error                 | 32               | 6.166        | 26764.13     | 29970.93 | 11885.04| 0.35%         | 2.97             | 0.91| 1.96          | 0.47        | 4.56        |

*Significant at 5% probability level,
** Significant at 1% probability level,
ns No significant
Table 3. The percentage of changes imposed by different light intensities and CO\(_2\) concentrations on biomass and WUE of lettuce in comparison with the control treatment (300 PPFD light intensity and 800 ppm CO\(_2\) concentration) after 40 days of growth

| Treatment              | Fresh weight | Dry weight | Leaf area | Leaf area index | Evapo-transpiration | WUE  |
|------------------------|--------------|------------|-----------|-----------------|---------------------|------|
| 75 PPFD, 400 ppm       | -74.73       | -79.12     | -62.92    | +77.57          | -39.5               | -71.28 |
| 75 PPFD, 800 ppm       | -75.86       | -73.04     | -55.28    | +73.73          | -39.62              | -62.35 |
| 75 PPFD, 1200 ppm      | -69.33       | -63.79     | -30.40    | +115.02         | -37.35              | -54   |
| 75 PPFD, 1600 ppm      | -57.91       | -57.80     | -27.57    | +73.13          | -37.98              | -42.74 |
| 150 PPFD, 400 ppm      | -61.53       | -61.15     | -50.18    | +29.64          | -28.68              | -51.73 |
| 150 PPFD, 800 ppm      | -16          | -47.67     | +15.51    | +147.37         | -27.29              | -40.45 |
| 150 PPFD, 1200 ppm     | -8.26        | -10.93     | +20.51    | +57.31          | -22.75              | -3.13  |
| 150 PPFD, 1600 ppm     | -31.15       | -19.12     | -2.31     | +21.83          | -37.58              | -10.19 |
| 300 PPFD, 400 ppm      | 0            | 0          | 0         | 0               | 0                   | 0     |
| (Control)              |              |            |           |                 |                     |       |
| 300 PPFD, 800 ppm      | +6.37        | +31.63     | +60.48    | +22.35          | +2.45               | +32.40 |
| 300 PPFD, 1200 ppm     | +54.74       | +147.87    | +88.69    | -14.12          | +21.06              | +98.53 |
| 300 PPFD, 1600 ppm     | +54.67       | +113.66    | +88.41    | -11.15          | +20.78              | +86.94 |
| 600 PPFD, 400 ppm      | -6.02        | +34.71     | -20.39    | -8.44           | -6.03               | +31.08 |
| 600 PPFD, 800 ppm      | +9.89        | +68.37     | +2.44     | -38.98          | +21.97              | +46.13 |
| 600 PPFD, 1200 ppm     | +19.51       | +147.67    | +77.93    | -71.1           | +29.68              | +84.19 |
| 600 PPFD, 1600 ppm     | +36.95       | +147.77    | +59.12    | -34.61          | +54.48              | +83.57 |

(A) 

(B)
Figure 2. Effect of different light intensities and CO\textsubscript{2} concentrations 400 (A), 800 (B), 1200 (C) and 1600 (D) ppm on dry weight of lettuce harvested at 10, 20, 30 and 40 days after application of treatments.
Figure 3. Effect of different light intensities and CO₂ concentrations 400 (A), 800 (B), 1200 (C) and 1600 (D) ppm on dry weight of lettuce harvested at 10, 20, 30 and 40 days after application of treatments.
Leaf area influenced by different light intensities and CO$_2$ enrichments

Leaf area of lettuce was considerably influenced by light intensity and CO$_2$ concentration (significant at 1% probability level) (Table 2). Largest leaf area was obtained in plants grown at 300 PPFD light intensity under 1200 ppm and 1600 ppm CO$_2$ concentrations. Leaf area reached to its maximum as a result of exposure to 1200 ppm CO$_2$ in all light intensities. In general, increasing light intensity and CO$_2$ concentration resulted in increase in the leaf area of lettuce plants (Figure 4C).

CO$_2$ enrichment did not affect SLA, while light intensity had a significant influence on SLA (Table 2). SLA decreased significantly by increasing light intensity (P < 0.01) (Table 2). There was a negative relationship ($R^2$=0.91) between light intensity and SLA. By increase in light intensity from 75 to 600 PPFD, SLA gradually decreased; the highest SLA was detected in 75 PPFD and the lowest SLA was obtained in plants that were grown under 600 PPFD (Figure 5).
Figure 5. Effect of different light intensities on specific leaf area (SLA) of lettuce plants

Stomatal morphology altered by light intensity and CO₂ enrichment

Stomata on the lettuce leaf that developed under 300 and 600 PPFD and 1600 ppm CO₂ concentration had shorter length in comparison with stomata that developed under standard condition. Except for 150 PPFD light intensity, under all other light intensities CO₂ enrichment negatively influenced the stomatal length. The shortest length of stomata was observed in plant grown under 600 PPFD light intensity and 1600 ppm CO₂ concentration (Figure 6A).

There was a significant interaction between light intensity and CO₂ concentration on the width of stomata and the width of its pore (significant at 1% probability level) (Table 2). The width of stomata and its pore aperture was decreased as a result of increasing CO₂ concentration under 300 and 600 PPFD light intensities (Figures 6B, C). Shortest pore aperture was detected under lowest light intensity and 1200 ppm CO₂, widest stomatal and pore widths were detected as a result of exposure to 400 ppm and 800 ppm CO₂ under 600 PPFD light intensity (Figure 6C). In plants that were grown under 300 PPFD, the stomatal density was the lowest compared to the stomatal density in the plants grown under other light intensities (Figure 6D).

Evapotranspiration and WUE increased by elevating light intensity and CO₂ concentration

Light intensity and CO₂ concentration strongly influenced evapotranspiration (significant at 1% probability level) (Table 2). Evapotranspiration increased at 300 PPFD and 600 PPFD by CO₂ enrichment (Figure 7A). Under highest light intensity, evapotranspiration increased in a CO₂ concentration-dependent manner. Evapotranspiration did not change by exposure to different CO₂ concentrations under 75 PPFD light intensity. Highest evapotranspiration belonged to plants exposed to highest light intensity and CO₂ concentration. In this experiment, evapotranspiration was not increased by CO₂ enrichment at lowest light intensity.

The result showed that the simple effects of light intensity and CO₂ enrichment significantly affected WUE of lettuce plants (significant at 1% probability level), while their interactions did not result in significant effect on WUE (Table 2). The percentage of variation indicated that WUE elevated by increasing light intensity from 75 to 600 PPFD (Table 3). Moreover, the effect of CO₂ enrichment on WUE is more than light intensity (Table 3). At 300 PPFD light intensity, WUE in plants that were grown under 1200 ppm was approximately 3.9 g L⁻¹ (Figure 7B) that was two times higher than WUE in plants that were grown under 400 ppm (Figure 7C).
(A) Stomata length (µm) vs. Light intensity (PPFD)

(B) Stomata width (µm) vs. Light intensity (PPFD)

(C) Stomata pore width (µm) vs. Light intensity (PPFD)
Figure 6. Effects of different light intensities and CO$_2$ concentrations on stomatal length (A), stomatal width, (B) pore width (C) and stomatal density (D) after 40 days of lettuce growth.
Plant growth parameters were significantly influenced by light intensity and CO\(_2\) enrichment (Table 2). Increasing light intensity and CO\(_2\) concentration resulted in best lettuce growth performance in 300 PPFD and 1200 ppm CO\(_2\). The reason why increasing light intensity and CO\(_2\) concentration resulted in a peak in biomass till a threshold level (300 PPFD light intensity and 1200 ppm CO\(_2\) concentration); in a way that more than this threshold level biomass was decreased, is related to gas exchange mechanism of lettuce response to light intensity and CO\(_2\) concentration. One of the most important effects of CO\(_2\) enrichment is decreasing stomatal conductance while on a same time increasing plant photosynthetic rate. It has been shown that rate of CO\(_2\) uptake in plants is particularly sensitive to CO\(_2\) concentration in the environment (Farquhar et al., 1980; Pearcy and Bjorkman, 1983). Proper range of light intensity promotes development of photosynthetic performance on the leaves (Prioul et al., 1980). Both high and low light intensities make an inefficient electron transport in the photosynthesis system (Fu et al., 2012). Thus, by application of high CO\(_2\) concentration, the leaf carbon balance will improve under low light intensities. It has been reported that the rate of photosynthesis is positively correlated with the CO\(_2\) concentrations under given light intensity (Pan et al., 2019). At the highest light intensity and CO\(_2\) concentration in the present study (600 PPFD and 1600 ppm CO\(_2\)) the lettuce fresh weight declined (Table 3). Although increasing light intensity will enhance the efficiency of CO\(_2\) utilization and increase the rate of photosynthesis and plant growth (Poudel and Dunn, 2017). As indicated before, the rate of photosynthesis cannot be further improved after certain intensity of light termed as the light saturation point, which is the highest intensity of light that a plant can use to boost its rate of net photosynthesis. Therefore, additional CO\(_2\) than the threshold levels is surplus and probably negatively influenced other plant processes. It seems increasing CO\(_2\) concentration and light intensity together helped to improve light saturation point and as a result of higher photosynthesis more biomass was produced by lettuce plants. In accordance with the obtained results, Pan et al. (2019) reported that increasing light intensity and CO\(_2\) concentration had a positive effect on growth and dry matter accumulation through improving the photosynthetic performance of the plants.

Leaf area of lettuce was also significantly altered by changes in light intensities and concentration of CO\(_2\). Increase in light intensity and CO\(_2\) enrichment resulted in expansion in lettuce leaf area. Leaf area under
high concentration of CO$_2$ and 300 PPFD light intensity reached to its maximum expansion (Table 3). However, it seems for the leaf area there was threshold levels for light intensity and CO$_2$ concentration. In this way, leaf area increased by increasing both light intensity and CO$_2$ concentration till 300 PPFD light intensity and 1200 ppm CO$_2$ concentration, but it declined in the highest light intensity (600 PPFD) and CO$_2$ concentration (1600 ppm). That reason is related to the rate of photosynthesis and different types of lettuce reaction to different light intensities (Pan et al., 2019). The plant response to light intensity and CO$_2$ enrichment is not linear (Hoittenschwiler and Korner, 1996). The previous studies showed that despite of positive effect of increasing light intensity and CO$_2$ concentration, the rate of photosynthesis would not further increase by plant exposure to very high light intensities and concentrations of CO$_2$ (Ho, 1977; Ito, 1978; Clough et al., 1981; Caporn, 1989; Sage et al., 1989). SLA was decreased by plant exposure to higher light intensities. This indicated that plants invest less biomass to develop leaf area due to absorption of enough light energy under higher light intensities, while invest more biomass to develop leaf area in order to receive enough light energy under low light intensities (Pan et al., 2019).

Stomata are the main paths for exchange of gases and water vapor between internal plant tissues and surrounding environment (van Meeteren and Aliniaeifard, 2016). It is well documented that the light intensity affects both stomatal closing ability and anatomy. Sensitivity of stomata depends on the light intensity and CO$_2$ concentration (Fanourakis et al., 2019A; James and Csiro, 1985). In this study, different light intensities and concentrations of CO$_2$ caused alterations in stomatal anatomical characteristics (Table 2). Alternation in stomatal characteristics due to leaf development under certain environmental conditions has also been previously reported (Savvides et al., 2012; Aliniaeifard and Van Meeteren, 2016; van Meeteren and Aliniaeifard, 2016; Asayesh et al., 2017; Fanourakis et al., 2019B). Stomatal development and its morphological characteristics are influenced by light intensity and CO$_2$ concentration during the plant growth (Lee et al., 2007). High stomatal conductance and leaf hydraulic conductance were detected in plants grown under high light intensity in comparison with those grown under lower light intensity (Sack, 2004). In agreement with the previous studies (Gorton et al., 1993; Thomas et al., 2004), our results showed alterations in stomatal characteristics due to exposure to different light intensities. We found a gradual increase in stomatal pore width with increase in light intensity at lower CO$_2$ concentrations but it slightly decreased in the high light intensities and lower CO$_2$ concentrations (Figure 6C). Increase in stomatal pore aperture can promote stomatal conductance (Lee et al., 2007). However, decrease in stomatal pore aperture due to limited light intensity could restrain photosynthesis by increasing diffusive resistance to CO$_2$ uptake (Lawson et al., 2010). CO$_2$ flows from the atmosphere to intercellular air spaces through the stomatal pores, and diffuses across the wall, plasmalemma, cytosol and finally to the chloroplast envelope for utilization in photosynthesis. The partial pressure of CO$_2$ in the intercellular air spaces is controlled by stomatal openings. Generally, as ambient CO$_2$ partial pressures increases, stomata tend to close (Jarvis et al., 1999).

WUE influenced by both anatomical (leaf area) and physiological (stomatal conductance) drivers (Giday et al., 2014). Based on the obtained results, biomass production increased by elevation in light intensity and CO$_2$ enrichment. Stomata are the main channels for the water vapor loss in the transpiration process (van Meeteren and Aliniaeifard, 2016); therefore, alterations in stomatal morphology can influence WUE of plant (Fan et al., 2013). Stomata are the main plant structure that control photosynthesis and transpiration, the two main gas exchange processes determining the WUE of the plants. Stomata as the gas exchanges portals control temperature and WUE of the plants (Li et al., 2017). Stomatal aperture is also one of the most important determinants of maximal stomatal conductance in plants (Franks et al., 2009). A reaction to light independent of CO$_2$ concentration was postulated by Heath and Russell (1954) to explain opening due to increased light intensity which occurred while the intercellular space CO$_2$ concentration was maintained at a low level.

Evapotranspiration is introduced as the loss of water to the atmosphere by the combined processes of evaporation from the soil and plant surfaces and transpiration from the plants (Aytek, 2008). It is essential to measure evapotranspiration for managing water resources in agriculture (Tabari et al., 2012). Furthermore, it is one of the most important parameters that explain water relationship between plant and the environment.
Stomatal conductance depends on the balance between internal and ambient CO$_2$ concentrations (Ci/Ca). This balance closely depends on the ambient CO$_2$ concentration. Elevated Ca through increasing Ci eventually leads to decrease in stomatal pore aperture and as a result increases WUE (Pazzagli et al., 2016). Furthermore, the growth parameters induced by increasing CO$_2$ concentration are directly related to water status in different plant species (Robredo et al., 2007). In the present study, WUE was increased in a light intensity- and CO$_2$ concentration-dependent manner. The stomata of plants that were exposed to higher light intensity and CO$_2$ concentration were closer than the stomatal opening of the plants exposed to lower light intensities and CO$_2$ concentrations, which resulted in higher WUE in plants exposed to higher light intensity and CO$_2$ concentration. In the present study, minimum stomatal pore aperture was detected in plants exposed to 300 PPFD and 1200 ppm CO$_2$ concentration, which resulted in maximum WUE (Table 3). The major effects of increased Ca will be increased CO$_2$ assimilation rate and decreased transpiration rate, the two responses leading to increased WUE (carbon gain/unit water lost). Although it may deduce somewhat contradictory that an increase in WUE are concomitant with an increase in growth of plants with limited water supplies (James and Csiro, 1985). It has been reported that CO$_2$ enrichment significantly enhances both WUE and photosynthesis while decreases evapotranspiration (Woodward et al., 2001). Therefore, alteration in stomata morphological characteristics as a result of exposure to elevated CO$_2$ concentration results in limited water loss but simultaneously provide the CO$_2$ demand for photosynthesis and improves WUE.

Conclusions

To optimize plant growth, it is important to establish proper environmental conditions such as lighting environment and CO$_2$ concentration. In commercial production, the supplemental lighting and CO$_2$ enrichment may heavily influence growth and quality of crops and as a result WUE. The result of present study showed that increasing light intensity and CO$_2$ concentration resulted in better growth performance in lettuce plants. The best growth performance for lettuce plants was obtained by growing them at 300 PPFD light intensity and 1200 ppm CO$_2$. Furthermore, stomatal length and stomata width decreased, while the WUE was increased by this treatment. Increasing light intensity and CO$_2$ concentration caused significant improvement in growth of lettuce plants. However, further increase above a threshold level (300 PPFD light intensity and 1200 ppm CO$_2$) imposed negative effects on the growth properties of lettuce plants.

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Conflict of Interests

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