



Influence of CO₂ Enrichment on Plant Growth and Physiology

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ABSTRACT

CO₂ enrichment can have many effects on plant growth and physiology, including: Increased photosynthesis- CO₂ is a key substrate for photosynthesis, so increased CO₂ levels can lead to higher rates of photosynthesis. Increased growth-Plants can grow faster when exposed to elevated CO₂ levels. This is due to increased growth in both roots and shoots. Increased nutrient uptake-Plants with enhanced root systems can absorb more nutrients from the soil. However, some studies have found that crops grown at higher CO₂ levels may have low levels of zinc and iron. Improved tolerance to stress-Plants grown with elevated CO₂ levels can be more tolerant to abiotic and biotic stresses. Improved yield and quality- Plants grown with elevated CO₂ levels can have higher yields and better quality. For example, tomatoes grown at higher CO₂ concentrations can have larger fruits. Improved utilization of light and water-Plants grown with elevated CO₂ levels can use light and water more efficiently. Decreased water use-Plants grown with elevated CO₂ levels can use less water. Lowered tissue concentrations of nitrogen and protein-Plants grown with elevated CO₂ levels can have lower concentrations of nitrogen and protein in their tissues. The effect of CO₂ on plant growth is strongly dependent on temperature. At relatively cold air temperatures, CO₂ enrichment can actually reduce plant growth.

KEYWORDS- CO₂ enrichment, plant growth, physiology, soil, nutrients

INTRODUCTION

The crops cultivated in greenhouses primarily consist of C₃ plants, including tomatoes and cucumbers (Sage, 2017). Due to their limited ability to adapt to CO₂ scarcity, C₃ crops exhibit greater sensitivity to fluctuations in CO₂ levels compared to C₄ and CAM plants (Long et al., 2015). Notably, C₃ crops respond positively to elevated CO₂ concentrations (Ainsworth and Long, 2020). For example, a moderate increase in CO₂ levels to 550 ~ 650 μmol mol⁻¹ can enhance the yield of various C₃ crops by an average of 18% (Ainsworth and Long, 2020). Additionally, CO₂ concentrations around 1000 μmol mol⁻¹ can boost the levels of soluble sugars and certain nutrients in leafy, fruit, and root vegetables by approximately 10% ~ 60% (Dong et al., 2018). Elevated CO₂ plays a crucial role in numerous physiological processes in C₃ crops, including photosynthesis, signaling pathways, organ development, and resistance to both biotic and abiotic stresses. Furthermore, CO₂ enrichment enhances yield and quality while improving the efficiency of light and water utilization (Zhang et al., 2015; Hu et al., 2021; Ahammed and Li, 2022). More comprehensive information can be found in reviews by Xu et al. (2015); Dong et al. (2018); Kazan (2018); Ahammed et al. (2021); Poorter et al. (2021); Roy and Mathur (2021), and Chaudhry and Sidhu (2022).[1,2,3]

Current CO₂ enrichment technologies

Atmosphere ventilation

Ventilation facilitates the exchange of heat and CO₂ between the interior and exterior of the greenhouse through natural ventilation methods such as roof and side windows, or through forced ventilation (Ishii et al., 2014; Yasutake et al., 2017). While ventilation can continuously provide CO₂ from the atmosphere into greenhouses, its primary function is often to regulate temperature. In colder geographical areas with limited ventilation, an additional supply of CO₂ becomes essential (Stanghellini et al., 2008). Furthermore, relying solely on ventilation is insufficient to sustain CO₂ levels around crops at ambient air concentrations (Pascale and Maggio, 2008). Crop yield is significantly more influenced by CO₂ levels when they are lower (below 450 μmol mol⁻¹) compared to when they are higher (Vermeulen, 2014).

Compressed CO₂

The direct provision of compressed CO₂ guarantees a consistent and clean airflow. Nevertheless, due to the elevated market price and transportation expenses, it is more frequently utilized as a supplement to other methods or in scientific investigations such as Free-Air CO₂ Enrichment (FACE) (Sánchez-Guerrero et al., 2005; Allen et al., 2020). Furthermore, compressed CO₂ must be accompanied by equipment for gas storage and pressure regulation, which typically occupies some space within greenhouses (Kuroyanagi et al., 2014; Poudel and Dunn, 2017; Li et al., 2018).

Carbonaceous fuel burning

When heating the greenhouse through the combustion of natural gas, coal, biomass, and other carbonaceous fuels, the CO₂ produced during these processes can be supplied to crops or captured and stored for later use (Vermeulen, 2014). As a relatively effective method for reducing carbon emissions and production costs, this technique is widely implemented in contemporary greenhouse production (Dion et al., 2011; Marchi et al., 2018). Additionally, ventilation is often minimized during heating, which enhances the effectiveness of CO₂ enrichment (Kläring et al., 2007). A significant drawback, however, is that in regions or seasons where heating is unnecessary, the combustion of fuel for CO₂ generation is not advisable.

Considering that the gas produced from the combustion boiler contains excessive heat and harmful gases, such as NO_x, SO₂, and CO, effective cooling and purification processes are critically needed (Roy et al., 2014; Li et al., 2018). Moreover, the timing and dosage requirements frequently do not align between CO₂ and heat, necessitating the use of collection and storage devices along with CO₂ flow controllers (Dion et al., 2011). Takeya et al. (2017) suggested a system to gather an adequate amount of CO₂ at night when the heating system is operational, allowing the gas to be released during the day when crops have a heightened demand for CO₂.

It is increasingly urgent to transition from carbon-based fuels to clean energy sources in order to mitigate carbon emissions. This includes options such as solar energy, hydrogen energy, geothermal energy, and even the utilization of industrial waste heat (Vermeulen, 2014; Marttila et al., 2021). Concurrently, the costs associated with production activities that generate carbon emissions have risen significantly. Consequently, greenhouses that extract CO₂ from heating systems are confronted with the challenge of identifying alternative techniques for CO₂ enrichment (Vermeulen, 2014).

Chemical reaction

The chemical reactions involving bicarbonate (like baking soda) and acid, as well as the decomposition through direct heating, are relatively inexpensive and rapid methods for quantitatively obtaining pure CO₂ (Syed and Hachem, 2019). The theoretical control of the CO₂ production rate is feasible; however, practical operations are complex, leading to substantial amounts of CO₂ being produced uncontrollably, which can be detrimental to plants (Poudel and Dunn, 2017). Additionally, ammonia bicarbonate is occasionally utilized as a raw material, which can yield by-products that serve as fertilizers. Nonetheless, there is a risk of ammonia gas poisoning, necessitating NH₃ filtration in such scenarios (Sun et al., 2016).

Compost fermentation

The microbial fermentation of carbon-rich agricultural waste to decompose and release CO₂ for crop production is regarded as a beneficial technology that enhances production, diminishes agricultural carbon emissions, and simultaneously reduces environmental pollution (Karim et al., 2020). However, there are stringent limitations regarding the C/N ratio, pH, temperature, materials, and other conditions (Jin et al., 2009; Karim et al., 2020). Technologies that incorporate crop residues and animal manure composting (CRAM) to augment CO₂ levels have been developed to enhance vegetable yield and quality (Jin et al., 2009; Karim et al., 2020). Furthermore, secondary fermentation products may also be repurposed as a CO₂ source (Liu et al., 2021). Necessary measures should be taken to deal with several weaknesses in compost fermentation, such as 1) associated unpleasant odors; 2) threat of ammonia poisoning (Li et al., 2018); 3) unstable rate of generated CO₂ (Karim et al., 2020); and 4) a larger space and more labor input requirements compared with other enrichment techniques (Tang et al., 2022).[4,5,6]

Control strategies for CO₂ enrichment

The efficiency of CO₂ utilization (CUE), which is defined as the ratio of the net photosynthetic rate to the rate of CO₂ supply, is influenced by several factors, including excessive supply, natural leakage, the sensitive growth conditions of plants, and various environmental and biological factors (Sánchez-Guerrero et al., 2005; Kuroyanagi et al., 2014; Li et al., 2018). Typically, the CUE values in greenhouses are below 60%, indicating that a significant quantity of CO₂ escapes into the surrounding atmosphere (Kozai, 2013; Kuroyanagi et al., 2014). Consequently, numerous efforts have been directed towards developing control strategies for CO₂ enrichment from multiple perspectives to enhance the CUE within greenhouse environments.

Spatial distribution

The consistency of environmental factors plays a crucial role in the effective and uniform management of greenhouse cultivation, whereas the uneven spatial distribution of CO₂ is a common issue in nearly all greenhouse operations (Li et al., 2018). The absence of adequate air circulation and the relatively slow diffusion rates result in extremely low CO₂ concentrations around the canopy, particularly in areas with high leaf density where CO₂ demand is greatest (Hidaka et al., 2022). Enrichment systems that utilize a single-point outlet tend to create a more uneven spatial distribution of CO₂, leading to significant waste and an inability to satisfy production requirements (Zhang et al., 2020). Therefore, it is necessary to incorporate conveying pipes with perforations around the leaves. Hidaka et al. (2022) implemented a pipe-delivered local CO₂ enrichment system in strawberry cultivation, resulting in increased yields while conserving CO₂ supply. Another viable alternative involves the use of internal airflow stirring devices (Boulard et al., 2017; Syed and Hachem, 2019).

Period Setting

There exist multiple modes within the period setting for CO₂ enrichment, including continuous enrichment throughout both day and night, during daylight hours, or exclusively in the morning or evening. Continuous enrichment throughout the entire day or night is typically employed in controlled environments for experimental purposes (e.g., Mamatha et al., 2014; Hu et al., 2021). It is evident that maintaining elevated CO₂ levels all day is highly energy-intensive and results in significant carbon emissions, particularly since carbon assimilation is generally most vigorous in the morning (Xu et al., 2014). More importantly, prolonged exposure to elevated CO₂ can lead to photosynthetic acclimation in crops (Wang et al., 2013). Consequently, methods that involve CO₂ enrichment solely in the morning, rather than throughout the entire day, have been investigated.

Treatments that elevate CO₂ levels only in the morning have been shown to enhance biomass accumulation and improve flower and fruit quality, often yielding results comparable to those achieved with continuous daytime enrichment (Caliman et al., 2009; Xu et al., 2014). Conversely, a similar approach involving intermittent CO₂ enrichment has been observed to hinder photosynthesis and yield in crops such as cotton, wheat, chrysanthemums, soybeans, and tomatoes (Mortensen et al., 1987; Bunce, 2012; Allen et al., 2020). Additionally, the impact of nighttime CO₂ enrichment remains ambiguous, potentially varying by species or cultivar (Baker et al., 2022).

Concentration Control

The differences in CO₂ concentration between the interior and exterior of the greenhouse (C_{in} - C_{out}) and the air exchange rate, primarily influenced by ventilation, are two critical factors that affect Carbon Use Efficiency (CUE), in addition to the inherent photosynthetic capacity of the crop (Kozai et al., 2015; Yasutake et al., 2017). When the internal concentration (C_{in}) is set at a higher level, such as 1000 µmol mol⁻¹, the CUE falls below 50% even in a greenhouse without ventilation, due to substantial CO₂ leakage (Kuroyanagi et al., 2014).

Moderate CO₂ enrichment control systems, which are based on the crop absorption rate or the difference between C_{in} and C_{out}, and maintain a Carbon Use Efficiency (CUE) close to 100%, have been shown to enhance the yield of cucumbers and tomatoes (Klaring et al., 2007; Kozai et al., 2015). Therefore, it is a viable and sustainable approach to maintain a moderate CO₂ concentration that is slightly above the ambient level, for instance, between 550 and 650 µmol mol⁻¹, while taking into account economic costs and environmental sustainability (Vermeule, 2014; Kozai et al., 2015). The yield and quality discrepancies when compared to crops grown under optimal CO₂ conditions may be mitigated by regulating other environmental factors and applying moderate environmental stresses (Kozai et al., 2015; Dong et al., 2018).

Notably, while there is a consistent finding of increased yield, the impact of elevated CO₂ on crop quality varies (Dong et al., 2018), indicating that the ideal CO₂ concentration should be tailored to specific production needs rather than adhering to a fixed value. In comparison to ambient CO₂ levels and lower CO₂ elevations (550 µmol mol⁻¹), the production of glucose and fructose is enhanced at higher CO₂ concentrations (700 - 1000 µmol mol⁻¹), although some amino acids and minerals may decrease (Högy and Fangmeier, 2009; Dong et al., 2018). The variations in health-promoting compounds and flavor components under increased CO₂ levels, such as flavonoids, lycopene, ascorbic acid, and carotene, are contentious across different vegetable crops, potentially due to the unique characteristics of various plant organs and the disruption of synthesis processes by other environmental factors (Mamatha et al., 2014; Dong et al., 2018; Hao et al., 2020).

Guidelines for Future CO₂ Enrichment

In addition to the necessity of enhancing yields and improving quality, the global agricultural production system is under significant pressure to minimize its carbon footprint in order to combat climate change. Although the photosynthesis process in crops primarily utilizes CO₂ as the inherent driving force of agriculture, protected agriculture across various nations and regions remains a process that is intensive in carbon emissions (Marttila et al., 2021; Northrup et al., 2021). Therefore, fully leveraging the crop's capacity for carbon fixation while integrating the strengths of diverse disciplines should be regarded as a sustainable approach to simultaneously address the challenges posed by global food production and climate change.

DISCUSSION

Assessing the Effects of CO₂ Enrichment on Photosynthesis

A multitude of research investigations have been undertaken to explore the impact of CO₂ enrichment on photosynthesis and plant growth. The findings consistently indicate that increased levels of CO₂ have a beneficial effect on photosynthetic rates, leading to enhanced biomass production. When plants are subjected to elevated concentrations of CO₂, they demonstrate a phenomenon referred to as the 'CO₂ fertilization effect.' This effect is characterized by accelerated photosynthesis rates, as the increased availability of CO₂ enables plants to synthesize more glucose, thereby generating additional energy to facilitate growth and development.

Positive Effects on Plant Productivity

The advantages of CO₂ enrichment go beyond just enhancing photosynthesis. It not only promotes increased glucose production rates but also improves various facets of plant physiology. For example, the heightened availability of carbon dioxide results in the upregulation of enzymes that facilitate photosynthesis, thereby optimizing the entire process. Furthermore, the phenomenon known as the 'CO₂ fertilization effect' leads to a significant rise in

biomass accumulation, yielding larger and more resilient plants. This enhancement in growth is especially beneficial in agricultural settings, where elevated crop yields can have a profound effect on food production and security.

One of the most notable advantages of CO₂ enrichment in studies of plant growth is the acceleration of overall plant development. When exposed to elevated CO₂ levels, plants exhibit increased rates of cell division and elongation, culminating in quicker growth and larger sizes. This growth enhancement is particularly beneficial in agricultural environments, as it directly correlates with increased crop yields and enhanced productivity.

The rapid plant growth seen with CO₂ enrichment can have extensive implications for food production and global food security. By creating a controlled environment with higher CO₂ concentrations, researchers and farmers can optimize conditions for crop growth and enhance the efficiency of photosynthesis, resulting in a more plentiful and sustainable food supply. While CO₂ enrichment promotes swift plant growth, it also affects the uptake and utilization of vital nutrients by plants. As CO₂ levels rise, plants may modify their nutrient uptake patterns, which can influence their nutritional content and overall quality.

For example, elevated CO₂ levels can result in a reduction of the relative concentration of specific plant minerals and nutrients. This occurrence is known as the "dilution effect." Despite the fact that plants may exhibit enhanced growth, the increase in mineral content may not occur at a corresponding rate, which could impact the nutritional quality of crops. Scientists are exploring methods to optimize nutrient management in environments enriched with CO₂ to mitigate this issue. By comprehending the intricate relationships between CO₂ enrichment and nutrient absorption, researchers aspire to create customized strategies that ensure crops retain their nutritional value while benefiting from accelerated growth.

Balancing Nutritional Content in Plants

Striking a balance between rapid growth and the preservation of optimal nutritional content is vital for the effective agricultural application of CO₂ enrichment. Although the increase in biomass is advantageous for higher yields, it is equally important to maintain the essential vitamins, minerals, and other nutrients that enhance the nutritional quality of crops. Researchers are striving to identify plant varieties that demonstrate resilience to nutrient imbalances in CO₂-enriched conditions. Furthermore, they are investigating the feasibility of sustainable nutrient supplementation strategies to guarantee that crops remain nutritious even in environments with elevated CO₂ levels.

Optimizing Nutrient Management

The integration of precision agriculture methods can play a vital role in enhancing nutrient management within CO₂-enriched settings. By customizing nutrient application to align with the distinct requirements of plants thriving in elevated CO₂ environments, farmers can achieve a harmonious balance between promoting growth and maintaining nutritional quality. Additionally, advancements in plant genetics and breeding may present opportunities to cultivate crops that are more suited to CO₂-rich conditions. By choosing plant varieties that excel under increased CO₂ levels without sacrificing nutrient content, agricultural practices can effectively and sustainably leverage the advantages of CO₂ enrichment.

CO₂ Enrichment: A Solution for Sustainable Agriculture

As the global population continues to expand, the demand for food rises correspondingly. Ensuring food security while preserving ecological integrity presents considerable challenges for contemporary agriculture. In this regard, CO₂ enrichment stands out as a viable solution for sustainable agriculture, providing innovative strategies to boost productivity and adapt to evolving environmental circumstances.

Addressing Food Security with CO₂ Enrichment

Sustainable agriculture seeks to fulfil current food production needs without jeopardizing the capacity of future generations to satisfy their own requirements. CO₂ enrichment plays a pivotal role in optimizing plant growth and enhancing crop yields. By increasing CO₂ levels in controlled environments, such as greenhouses, farmers can take advantage of the CO₂ fertilization effect to elevate photosynthesis rates and achieve greater biomass production. The improved growth resulting from CO₂ enrichment leads to a more substantial potential for food production. Given the continuously growing population, this becomes essential in addressing the escalating global food demand and ensuring food security for everyone. (13,14,15)

Addressing the Global Food Demand

The United Nations projects that the worldwide population will hit 9.7 billion by the year 2050. This rapid growth necessitates innovative agricultural methods to generate sufficient food to sustainably nourish the global populace. CO₂ enrichment presents a viable solution to reconcile the disparity between food supply and demand, ensuring that adequate food resources are accessible to all.

By establishing CO₂-enriched conditions in controlled agricultural environments, farmers can grow crops at accelerated rates, resulting in enhanced yields and greater food availability. This method complements conventional farming techniques, providing a practical approach to boost agricultural output without the need to expand farmland.

Sustainable Agricultural Practices

Sustainable agriculture transcends simple productivity; it involves conscientious resource management and ecological conservation. CO₂ enrichment is consistent with these ideals by maximizing resource efficiency in plant growth. Since CO₂-enriched environments typically experience lower water loss due to stomatal regulation, plants grown in these conditions require less water for their growth and development. This efficiency in water usage is vital, particularly in areas grappling with water shortages or erratic climate conditions. CO₂ enrichment aids in sustainable water management in agriculture by reducing overall water consumption.

Embracing a Sustainable Future

The enrichment of CO₂ in studies related to plant growth represents a promising pathway for sustainable agriculture. By enhancing photosynthesis and promoting plant growth while emphasizing resource efficiency, this method provides a feasible response to the pressing issues of food security and the adaptation to climate change. As researchers delve deeper into the effects of CO₂ enrichment on different crop species and their growing conditions, the prospects for sustainable agriculture become increasingly clear. The incorporation of CO₂ enrichment into agricultural practices, alongside other sustainable farming methods, lays the groundwork for a future characterized by abundant and environmentally responsible food production.

Climate change presents considerable challenges to agriculture on a global scale. Increasing temperatures, severe weather events, and altered precipitation patterns can hinder crop development and diminish yields. Within this framework, CO₂ enrichment stands out as a potential adaptive measure that may assist plants in adjusting to fluctuating environmental conditions. As atmospheric CO₂ levels rise due to anthropogenic activities, plants in natural settings encounter elevated concentrations of CO₂. This ongoing phenomenon can influence plant physiology and growth patterns. Conversely, in controlled environments where CO₂ enrichment is applied, plants are deliberately subjected to higher CO₂ levels, allowing them to adapt more efficiently.

Rising CO₂ Levels and Their Impact

The growing concentration of CO₂ in the atmosphere poses a "double-edged sword" for vegetation. On one side, increased CO₂ can enhance photosynthesis and promote growth. Conversely, it also influences other facets of plant physiology, including nutrient absorption, interactions between plants and pollinators, as well as responses to pests and diseases. In natural settings, escalating CO₂ levels may result in ecological imbalances and impact biodiversity. While certain plant species might thrive due to the heightened CO₂, others could be adversely affected, leading to changes in plant communities and ecosystem dynamics.

Ensuring Crop Resilience

In controlled settings where CO₂ levels are enriched, researchers can investigate and manipulate the effects of rising CO₂ on various crops. By examining the responses of different plant species and cultivars to elevated CO₂, they can acquire knowledge about potential ecosystem alterations and formulate strategies to sustain crop resilience amid climate change.

The adaptive characteristics of CO₂ enrichment enable farmers to grow crop varieties that are better equipped to face the challenges brought about by climate change. By selecting plants that exhibit greater resilience to fluctuating environmental conditions, farmers can enhance their capacity to achieve stable yields and ensure food production.

Mitigating Potential Risks

Although CO₂ enrichment presents promising advantages, it is crucial to acknowledge the possible risks and challenges. In open environments, uncontrolled CO₂ emissions can intensify the greenhouse effect, worsening climate change and its related consequences. This highlights the necessity for responsible CO₂ management and global efforts to diminish greenhouse gas emissions.

Furthermore, it is essential to comprehensively understand the interactions between CO₂ enrichment and various environmental factors. The intricate nature of ecological systems necessitates a careful examination of how increased CO₂ levels may affect nutrient cycling, soil health, and the interactions between plants and insects. In studies focused on plant growth, CO₂ enrichment plays a crucial role in enhancing photosynthesis and accelerating growth rates. By creating a controlled environment with elevated CO₂ concentrations, researchers and agricultural practitioners can leverage the 'CO₂ fertilization effect' to achieve improved yields and foster sustainable agricultural practices. Integrating CO₂ enrichment into a holistic strategy for food security and climate change adaptation presents significant potential for a resilient and productive agricultural future.

RESULTS

Lettuce (*Lactuca sativa* L.) is a crop of considerable commercial significance and serves as an excellent candidate for cultivation in a plant factory utilizing artificial lighting (PFAL). To investigate the effects of CO₂ enrichment (e CO₂) on the growth of lettuce, light-use efficiency (LUE), and various growth indicators were meticulously evaluated. Three distinct CO₂ concentrations (CO₂) were employed: ambient CO₂ (serving as the control, approximately 400 µmol mol⁻¹), roughly double the ambient level (DA- CO₂, 800 ± 50 µmol mol⁻¹), and approximately quadruple the ambient level (QA- CO₂, 1600 ± 50 µmol mol⁻¹) over a duration of 30 days. The findings revealed that plant height, stem diameter, leaf count, root length, leaf width, and maximum

leaf area all exhibited positive correlations with CO₂, demonstrating that growth was significantly enhanced in the e CO₂ treatments compared to ambient CO₂. Additionally, the daily average assimilation rate (DAAR), average dry weight growth rate (GRdw), and overall yield were notably higher for plants cultivated under DA- CO₂ and QA- CO₂ compared to those grown under ambient CO₂ (with DAAR increasing by 25.45% and 42.27%, GRdw rising by 28.76% and 37.55%, and yield increasing by 33.65% and 44.16%, respectively).

The effects of DA- CO₂ and QA- CO₂ on various parameters of lettuce growth were measured. Over a 30-day period, elevated CO₂ positively influenced several growth metrics, including height, stem diameter, leaf count, root length, leaf length, and maximum leaf area. The effects were notably more significant in the QA- CO₂ treatment. We concluded that the CO₂ levels utilized in this study positively impacted these growth indicators of lettuce throughout the duration of the experiment. Likewise, the leaf area and dry weight of grafted pepper (*Capsicum annuum* L.) under elevated CO₂ were found to be greater than those under ambient CO₂ (Jang et al., 2014), and Singh et al. (2013) reported that elevated CO₂ increased both the number of leaves and leaf area in cotton (*Gossypium hirsutum* L.). Additionally, elevated CO₂ significantly enlarged the leaf size of strawberry (*Fragaria ananassa* Duch.) (Li et al., 2020). In our investigation, we noted a higher number of leaves and a greater maximum leaf area under the DA- CO₂ and QA- CO₂ treatments compared to ambient CO₂ at harvest, aligning with the findings of Meng et al. (2014), who noted that maize (*Zea mays* L.) roots exhibited rapid growth early in their study under elevated CO₂, followed by a slower growth rate.

Photosynthetic pigments, which serve as the energy-absorbing components, are essential for the process of photosynthesis. Our findings indicated that there was no significant difference in Chl a, Chl b, and total Chl levels. Wujeska-Klaue et al. (2019) similarly reported a reduction in total Chl concentration when exposed to CO₂. An experimental study conducted by Lin et al. (2011) demonstrated the absence of a significant impact of CO₂, revealing that the carotenoids in cowpea (*Vigna unguiculata* (L.) Walp. plants cultivated at CO₂ concentrations of 800 and 1,600 µmol CO₂ m⁻³ were not significantly different from those grown in ambient CO₂ conditions. Conversely, Juknys et al. (2011) discovered that elevated CO₂ did not influence the levels of photosynthetic pigments in various agriculturally significant plants. Du et al. (2014) found a negative correlation between the Chl a, Chl b, and total chlorophyll content in seedlings of *Quercus variabilis* Blume. and [CO₂] levels. In a microalgal study, elevated CO₂ did not affect Chl a or carotenoid concentrations, but it did lead to a reduction in Chl b (Liu et al., 2018). Nonetheless, there was a tendency for increased carotenoids, Chl a, and Chl b under the DA- CO₂ treatment, while the QA- CO₂ treatment exhibited a trend of reduced carotenoids and lower levels of Chl a and Chl b compared to ambient CO₂. It has been proposed that higher [CO₂] levels diminish chlorophyll content, whereas optimal [CO₂] levels enhance carotenoid production. Our observation of a slight increase in carotenoids alongside a decrease in chlorophyll aligns with this hypothesis. Supporting this notion, Cave et al. (2010) noted a decrease in the Chl a to Chl b ratio in *Trifolium subterraneum* L. under elevated [CO₂], which corroborates our findings and may be attributed to high [CO₂] levels accelerating chlorophyll degradation (de la Mata et al., 2012). Additionally, Aranjuelo et al. (2015) reported that elevated CO₂ resulted in a 140% increase in plant shoot biomass and enhanced the dry weight of grain in durum wheat (*Triticum turgidum* Desf.). In a similar vein, we noted that the fresh weight of the plant, the average growth rate, and the dry matter of the shoots all exhibited an increase with elevated CO₂ levels. The trend observed in the dry weight of the roots and the ratio of root to shoot dry matter experienced some modification; however, plants grown in ambient CO₂ conditions displayed the lowest root dry matter and the least root to shoot dry matter ratio. In additional studies, lettuce subjected to 1,000 µmol mol⁻¹ CO₂ demonstrated a 72% increase in shoot mass compared to lettuce cultivated under 200 µmol mol⁻¹ CO₂ (Becker & Kläring, 2016), which aligned with our findings. The rise in CO₂ concentration resulted in enhanced growth by augmenting the leaf mass per area (Ishizaki et al., 2003).

The Daily Accumulation Rate of Dry Matter (DAAR) for lettuce rose from 5 to 25 days in the elevated CO₂ environment, subsequently declining after 25 days. Following 20 days, the DAAR pattern across treatments stabilized and showed a positive correlation with CO₂ levels, consistent with the principles of dry matter accumulation in lettuce. Furthermore, the treatment patterns remained consistent at harvest, in accordance with the established rule of changes in dry weight growth rate (GRdw). The average DAAR for plants in the DA- CO₂ and QA- CO₂ groups, evaluated throughout the growth period, increased by 25.45% and 42.27%, respectively, in comparison to the ambient CO₂ group. We concluded that elevated CO₂ significantly enhanced the DAAR of lettuce. The relatively modest increase in GRdw may have resulted from the lettuce's adaptation to the conditions present under elevated [CO₂].

The Light Use Efficiency (LUE) across all treatments varied throughout the experimental duration; however, we calculated the average LUE for the entire growth period. The averages for plants cultivated under DA- CO₂ and QA- CO₂ increased by 28.51% and 40.12%, respectively, when compared to ambient CO₂. Elevated CO₂ improved the LUE of lettuce and was positively correlated with (CO₂). Additionally, the overall trend in LUEs prior to 25 days was an increase, followed by a significant decline by 30 days, likely due to the enhancement of the photosynthetic capacity of the lettuce leaves during the vigorous growth phase. According to the research, the highest biomass was observed at a CO₂ concentration of 1,600 µmol mol⁻¹; however, when compared to 800 µmol mol⁻¹, the CO₂ input at 1,600 µmol mol⁻¹ was twice as much, yet the biomass did not increase proportionally. Consequently, the more cost-effective concentration appears to be 800 µmol mol⁻¹. In a Plant Factory with Artificial Lighting (PFAL), it remains to be determined whether 800 µmol mol⁻¹ is indeed the most economical concentration, necessitating further investigation into the gradient refinement. This study was conducted in climate chambers with limited space, which differ from actual PFALs and greenhouses. Nevertheless, this limitation is unlikely to alter the observed trend regarding the impact of CO₂ on plant growth. Thus, it can be reasonably anticipated that an increase in atmospheric (CO₂) may enhance the growth rate of crops to a certain degree.[17,18,19]

CONCLUSION

DA- CO₂ and QA- CO₂ during the growth of lettuce facilitate morphogenesis, thereby establishing a favorable basis for an increased daily average assimilation rate compared to ambient CO₂ levels. Both the average dry weight growth (GRdw) and biomass accumulation exhibited an increase with elevated CO₂ levels. This suggests that enhanced CO₂ could positively influence lettuce yields by promoting growth. However, it is important to note that

higher concentrations do not necessarily equate to better outcomes. The CO₂ input at 1,600 $\mu\text{mol mol}^{-1}$ is double that of 800 $\mu\text{mol mol}^{-1}$, yet the dry matter yield does not reflect this doubling, indicating that while CO₂ levels are higher, the efficiency of dry matter production is reduced, making 1,600 $\mu\text{mol mol}^{-1}$ less economical. Comparatively, while 1,600 $\mu\text{mol mol}^{-1}$ yielded the highest biomass, 800 $\mu\text{mol mol}^{-1}$ proved to be the most cost-effective. The Light Use Efficiency (LUE) experienced a significant decline at 30 days, consistent with the observed pattern of Daily Average Assimilation Rate (DAAR), suggesting that growth decelerated after 25 days, thus indicating that harvesting lettuce at 25 days would be prudent. Although the results indicated a slight reduction in chlorophyll content, the notable increase in leaf number and maximum leaf area could enhance the photosynthetically effective leaf area. There was a significant increase in the dry matter accumulation of lettuce, along with a marked improvement in LUE efficiency. Therefore, the findings of the research suggested that higher CO₂ levels could positively impact the growth rate, yield cultivation, and LUE of lettuce.[20]

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