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ORIGINAL ARTICLE

Effect of CO_2 Supply on Lettuce Growth

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Abstract

This study was conducted to investigate the effects of CO_2 supplement on growth and quality in greenhouse lettuce cultivation. When CO_2 was supplied at 1,500 ppm in lettuce cultivation, overall growth parameters such as number of leaves, leaf area, plant length, fresh weight, and dry weight were superior compared to those of the control group. While there was no significant difference in relative growth rate due to CO_2 supplement, an increase in leaf area index was observed with CO_2 usage.

Furthermore, although there was no significant difference in the content of water-soluble vitamins such as Vitamin C, B1, B2, B5, and B6 due to CO_2 supplement, the Vitamin B3 content in the CO_2 treatment group was 0.5 mg/kg higher than in the control group.

Therefore, the use of CO_2 in lettuce cultivation resulted in increased yield and promoted growth, enabling early harvesting.

Key words : Chlorophyll, CO_2 supply, Growth, Leaf area index, Relative growth rate

1. Introduction

Lettuce (*Lactuca sativa* L.) is a 1~2 year old herb belonging to the Asteraceae family, originating in Europe, and is a low-temperature crop with an optimal growth temperature of 15-20℃. Lettuce cultivation in Korea mainly involves leaf lettuce. On the other hand, the United States and Japan mainly cultivate head lettuce, and butterhead lettuce cultivation prevails in Europe.

Lettuce is composed of 95% water and is rich in various minerals such as proteins, sugars, calcium, phosphorus, as well as vitamins. It is beneficial for patients with constipation and anemia, and it holds the highest market share among all leafy vegetables used for wraps (RDA, 2018). In particular, red lettuce is known to contain higher levels of antioxidants and functional components compared to green lettuce (Lee et al., 2010).

In 2022, the cultivation area of lettuce in Korea was 3,913 hectares, with greenhouse cultivation accounting for the majority at 3,070 hectares (KOSIS, 2022). In 2020, the production value of greenhouse-grown lettuce was 26.5 billion Korean won, and the export amount is about $1,300 (RDA, 2021).

Carbon is an essential element constituting crops, and in low concentrations of CO_2 environments, photosynthesis is impaired while photorespiration increases, leading to poor growth (Sage and John, 2001; Bok et al., 2021). Especially at CO_2 concentrations below 150

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ppm, plant growth is significantly impaired, impacting fruit yield (Nelson, 1992).

Optimal CO_2 concentrations promote positive physiological responses such as increased leaf area, biomass, and yield in crop cultivation (Nederhoff et al., 1992; Goo et al., 2022; Lee et al., 2022; Lim et al., 2022). However, excessively high concentrations can lead to the closure of stomata, resulting in a decline in photosynthetic rate and causing issues in the hormonal system of plant (Lee et al., 2018).

The efficacy of CO_2 treatment varies depending on the type of crop, cultivar, maturity stage, and cultivation conditions (Arp, 1991). In recent years, with the increasing prevalence of venlo- type greenhouses in Korea, the use of CO_2 has been expanded in the cultivation of greenhouse vegetables such as European-style tomatoes, peppers, strawberries, aiming for high quality and increased yield.

Lettuce price often surge during the summer, primarily due to the challenging cultivation conditions caused by severe droughts along with prolonged periods of high temperatures in June and July. In response to such abnormal weather conditions, there is a growing interest in plant factories that enable stable lettuce cultivation (Lee, 2019).

From this perspective, this research was conducted to investigate the effects of supplying CO_2 on the growth and quality of lettuce cultivation, aiming to establish a stable production technology for lettuce.

2. Materials and Methods

2.1. Conducted cultivar and cultivation conditions

The cultivar of the lettuce used in this experiment was 'Yeolpung jeokchima' (Kwonnong, Chungcheongbuk-do, Korea). The experiment was conducted in a 40 m^2 venlo-type glass greenhouse, and pot cultivation was carried out with elevated benches installed. Sowing was done with 'Yeolpung jeokchima' (Kwonnong Seed Co.) in a 126-cell plug tray, and after 18 days of seedling cultivation, lettuce with at least two true leaves was transplanted into 1/5,000 Wagner pots on September 11th. For the soil, horticultural soil (Farmhannong, Seoul, Korea) was used and after filling 80% of the Wagner pots, the crops were transplanted. During the cultivation period, after diluting 400 times, a total of 50 ml of nutrient solution (Mulpurae type 1, Dae-yu Co., Korea) was supplied through a drip tube per day by using an automatic timer.

The pH of the nutrient solution was 5.6, with an electrical conductivity (EC) of 1.4 dS m^{-1}. The daytime and nighttime temperatures in the greenhouse were set at 20°C for cultivation. Additionally, to ensure proper temperature management during the experimental period, temperature and humidity were monitored by installing a temperature and humidity recorder (TR-72Ui, TSD Corporation, Japan) at a height of 50 cm above the plants from September 12th to November 11th.

2.2. CO_2 supply conditions

In order to test the growth response by applying CO_2 to lettuce cultivation, growth was examined between the treatment group where 1,500 ppm of CO_2 was applied and the control group (360 ppm) that was not treated with additional CO_2 during the entire growth period from planting to harvest. The CO_2 supply was provided in the form of bombe using liquid carbon dioxide with an on/off function from 9 am. to 5 pm.

2.3. Lettuce growth test by CO_2 supply

The growth of lettuce was investigated at 15, 30, 45, and 60 days after transplantation. The following items were investigated: number of leaves, leaf area, leaf length, leaf width, plant height, root length, fresh weight, dry weight, relative growth rate, and leaf area index. The investigation method involved sampling three plants per repeat, and number of leaves was investigated by counting leaves with an area of 1 cm^2 or more. Leaf area was measured by using a leaf area meter (LI-3000, LI-Co., USA).

Leaf length and width were measured on the third expanded normal leaf. Plant height was measured at the highest point of the above-ground part, while root length was measured by washing the roots with water to remove soil and measuring the longest part of the roots. Fresh weight was measured as the weight of the plant by electronic balance (AX2202KR/E, OHAUS Co., Ltd., Dajeon-shi, Korea) while dry weight was measured after drying at 70°C for 24 hours using thermal dryer (OF3-30W, JEIO TECH Co., Ltd., Dajeon-shi, Korea). Relative growth rate and leaf area index were calculated using the following formulas.

$$RGR = \frac{\mathrm{Ln}(W_a) - \mathrm{Ln}(W_b)}{t_a - t_b}$$

RGR; Relative growth rate

W_a : Dry weight of plant at t_a after planting (g)

W_b : Dry weight of plant at t_b after planting (g)

$t_a - t_b$: change in time

$$LAI = \frac{A_t}{A_a}$$

LAI; Leaf area index

A_t : leaf area (cm^2)

A_a : ground area (cm^2)

2.4. Quality testing of lettuce by CO_2 supply

Chlorophyll and anthocyanin contents were examined at 15, 30, 45, and 60 days after transplantation, while soluble vitamins, macro nutrients, and trace nutrients were examined on the 60th day after transplantation. Quality assessment of lettuce was conducted by sampling three plants per repeat.

The chlorophyll content was determined by measuring three points on the third fully expanded true leaf by using a chlorophyll meter (SPAD-502, Minolta Co., Ltd., Japan). After determining chlorophyll content, we obtained the average value of the chlorophyll content.

Anthocyanin extraction was performed on 1 g of lettuce obtained from each treatment using freeze-drying methodology. The frozen sample was homogenized by grinding in a mortar with 10 ml of a solution consisting of 85% ethanol and 15% 1.5 N HCl. Subsequently, homogenate was centrifuged at 5,000 rpm for 10 minutes (MICRO 17TR, Hanil, Korea), and the supernatant was collected and filtered through filter paper (Whatman No.2). The supernatant was diluted for 15 minutes by combining Potassium chloride buffer solution (0.025 M, pH 1.0) and Sodium acetate buffer solution (0.4 M, pH 4.5) in a 1:1 (v/v) ratio. After dilution, absorbance was measured at 520 nm and 700 nm by using a Spectrophotometer (Optizen 3220UVbio, MECASYS Co., Ltd, Dajeon-shi, Korea), and the anthocyanin content was determined. Absorbance (A) and anthocyanin content were calculated as follows.

A (Absorbance)=(A520-A700)pH1.0-(A520-A700)pH4.5

Anthocyanin content (mg/L)=(A × MW × DF × 1000)/(ε × 1)

MW : molecular weight of cyanidine-3-glucoside=449.2

DF : dilution factor

ε : molar absorptivity=26900

The water soluble vitamins, which are functional components, including vitamin B1, B2, B3, B5, B6, and C were measured. Lettuce harvested on the 45th day after transplantation was freeze-dried, and 50 mg was collected. After adding Internal standard (1 mg/ml) to lettuce sample, a solution composed of 650 μl of methanol, 700 ㎕ of 10 mM ammonium acetate, and 50 ㎕ of 0.1% BHT was added to the lettuce sample.

The sample was vortexed for 5 minutes and then subjected to sonication at room temperature for 5 minutes using an Ultrasonicator (2510RDTH, Bransonic®, Danbury, USA). Subsequently, centrifugation at 4,000 rpm for 5 minutes was conducted by using a centrifuge (MICRO 17TR, Hanil, Korea) to obtain the supernatant. The supernatant was extracted through a 0.45 μm PTFE syringe filter (SMI-Lab Hut Co., Ltd, Maisemore, UK). The extracted solution was quantitatively analyzed by using LC-MS/MS (Santos et al., 2012).

The macro nutrient and trace nutrient contents of lettuce, including total nitrogen (T-N), P, K, Ca, Mg, Mn, and Zn, was investigated after drying the harvested lettuce at 70°C for over 48 hours. Total nitrogen was measured by using the Kjeldahl method (Bremner, 1965), and phosphorus (P) was measured by using spectrophotometer at a wavelength of 470 nm by using the colorimetric method. Cations such as Ca, K, Mg, Mn, and Zn were measured by using an Atomic Absorption Spectrometer. The analysis method followed the guidelines outlined in the Rural Development Administration's Soil and Plant Analysis Method (NIST, 2000).

2.5. Statistical analysis

Statistical analysis was performed by using the SAS program (Version SAS 9.4, SAS Institute Inc., Cary, NC, USA), and the Least Significant Difference test was conducted at a confidence level of 95%.

Table 1. Effect of CO_2 supply treatment on number of fresh weight and dry weight for various growth state of 'Yeolpung jeokchima' lettuce at 20℃ in greenhouse

CO_2 supply (ppm)	Fresh weight (g/plant)			Dry weight (g/plant)		
	Shoot	Root	Total	Shoot	Root	Total
			0 days after transplanting			
Control	0.32	0.23	0.55	0.00	0.00	0.01
1,500	0.32	0.23	0.55	0.00	0.00	0.01
LSD[z]	-	-	-	-	-	-
			15 days after transplanting			
Control	16.22	16.16	17.83	0.61	0.08	0.70
1,500	18.01	17.57	19.76	0.73	0.11	0.84
LSD	NS	NS	NS	0.05	0.01	0.05
			30 days after transplanting			
Control	75.88	9.79	85.67	3.39	0.54	3.92
1,500	97.42	7.05	104.46	3.53	0.99	4.52
LSD	15.20	NS	16.92	NS	0.39	0.50
			45 days after transplanting			
Control	124.22	15.48	139.70	6.19	1.29	7.47
1,500	131.89	16.94	148.83	6.65	1.52	8.18
LSD	NS	NS	NS	NS	NS	NS
			60 days after transplanting			
Control	155.21	19.33	174.54	9.86	1.67	10.93
1,500	335.57	15.31	350.89	13.12	2.15	15.27
LSD	24.65	3.88	25.51	3.55	NS	3.78

[z] Means separation within columns of each growth stage by least significant difference (LSD) test at $P = 0.05$

Fig. 1. Changes in growth of 'Yeolpung jeokchima' lettuce seedling by CO_2 treatment various growth stage after transplanting.

3. Results and Discussions

3.1. Effect of CO_2 supply on lettuce growth

Recently, with the increase in venlo-type greenhouses equipped with advanced environmental control systems, tomato and pepper farmers are supplying CO_2 to enhance yield and promote growth (Lee et al., 2018). Table 1 presents the results of investigating the effects of CO_2 supply on the growth of lettuce, which is leafy vegetables.

When supplying CO_2 during lettuce cultivation, better aboveground growth, including number of leaves, leaf area, leaf length, leaf width, and plant length, was observed compared to the control group (360 ppm). This trend was maintained throughout the entire growth period of 60 days (Fig. 1).

Considering that on the 60th day after transplantation, the number of leaves in the CO_2 treated group was 50.9, while it was 31.2 in the control group, it can be concluded that CO_2 treatment resulted in an increase in number of leaves.

Additionally, the leaf area in the CO_2 treated group was 4852.0 cm^2, whereas it was 3598.65 cm^2 in the control group, indicating that the leaf area in the CO_2 treated group was approximately 1.35 times higher than that in the control group. This suggests that CO_2 treatment promoted the growth of leaf epidermal tissues, leading to an increase in number of leaves and leaf area (Taylor et al., 2001). On the other hand, there was no significant difference in plant height and leaf width between the CO_2 treated and control groups (Fig. 1).

On the 60th day after transplantation, there was a significant difference in the fresh weight and dry weight of lettuce due to CO_2 supply (Table 1). The aboveground fresh weight in the CO_2 treated group was 335.57 g, and the aboveground dry weight was 13.12 g, significantly higher than the control group's 155.21 g and 9.86 g, respectively. Additionally, the belowground dry weight in the CO_2 treated group was about 1.3 times higher than that in the control group.

It has been reported that in crop cultivation, the fresh weight and dry weight of plants increase when CO_2 supply concentration exceeds 1,000 ppm (Arp, 1991). In the case of lettuce, supplying CO_2 at a concentration of 1,500 ppm also resulted in an increase in both aboveground fresh weight and dry weight. Additionally, Fierro et al.(1994) found that CO_2 supply led to an increase in belowground fresh weight and belowground dry weight, although the effects among different CO_2 treatment concentrations were not significant.

In this research, enhancement of biomass production is presumed to be influenced by various factors including CO_2 treatment concentration, light intensity, and other cultivation environments and it was observed that CO_2 supply resulted in an increase in biomass production (Table 1).

Relative growth rate reflects a plant's capacity for biomass production over a specific period and is related to the individual's growth rate. In this study, there was no significant difference in relative growth rate between the CO_2 treated group and the control group (Table 2).

In a similar study investigating the effect of CO_2 supply on the relative growth rate of water parsley cultivation, it was reported that although the relative growth rate was higher with initial CO_2 supply, there was no significant difference compared to the control group after 3 weeks (Jung et al., 2022). Additionally, in water parsley supplied with CO_2, it was found that there was no significant difference in relative growth rate between the CO_2 treated group and the control group throughout all growth periods (Jung et al., 2022). Therefore, considering

Table 2. Effect of CO_2 supply treatment on relative growth rate (RGR) and leaf area index (LAI) for various growth state of 'Yeolpung jeokchima' lettuce at 20℃ in greenhouse

CO_2 supply (ppm)	RGR ($g \cdot g^{-1} \cdot day^{-1}$)	LAI ($cm^2 \cdot cm^{-2}$)
0 days after transplanting		
Control	-	0.04
1,500	-	0.04
LSD[z]	-	-
15 days after transplanting		
Control	0.05	1.99
1,500	0.05	2.17
LSD	NS	NS
30 days after transplanting		
Control	0.22	9.28
1,500	0.25	9.78
LSD	NS	NS
45 days after transplanting		
Control	0.24	12.19
1,500	0.24	12.86
LSD	NS	NS
60 days after transplanting		
Control	0.23	17.99
1,500	0.48	24.26
LSD	NS	3.00

[z] Means separation within columns of each growth stage by least significant difference (LSD) test at $P = 0.05$

both this study and previous research, it can be concluded that the effect of CO_2 supply on the relative growth rate in leafy vegetable cultivation is not significant.

Leaf area index (LAI) represents the total leaf area per unit area, and it showed an tendency of increasing throughout the entire growth period, with the CO_2 treated group higher than that of the control group. On the 60th day after transplantation, the leaf area index was 24.26 in the CO_2 treated group and 17.93 in the control group, indicating that the CO_2 treated group was 1.35 times higher than the control group (Table 2).

When atmospheric CO_2 concentration is high, it enhances photosynthesis in crops, leading to increased yield and improved quality. Therefore, CO_2 supply not only increases biomass and leaf area index in lettuce production but also enables early harvesting, consequently making early market distribution possible.

3.2. Effect of CO_2 supply on lettuce quality

In lettuce cultivation with CO_2 treated, the chlorophyll and anthocyanin contents were investigated (Table 3). The chlorophyll content in the CO_2 treated group was consistently higher than that in the control group throughout all growth periods, although the statistical difference was not significant. The chlorophyll content in the control group showed an tendency of increasing as the growth days passed. In contrast, the chlorophyll content in the CO_2 treated group remained relatively constant at 19 SPAD or higher throughout the entire growth period. This aligns with the findings of Li et al.(2019), which suggested that CO_2 treatment delays

Table 3. Effect of CO_2 supply treatment on chlorophyll and anthocyanin content for various growth state of 'Yeolpung jeokchima' lettuce at 20℃ in greenhouse

CO_2 supply (ppm)	Chlorophyll (SPAD unit)	Anthocyanin content (mg/100g)
	0 days after transplanting	
Control	-	0.10
1,500	-	0.10
LSD[z]	-	-
	15 days after transplanting	
Control	16.76	0.13
1,500	19.45	0.20
LSD	1.16	0.03
	30 days after transplanting	
Control	18.46	0.13
1,500	19.60	0.22
LSD	NS	0.05
	45 days after transplanting	
Control	19.11	0.51
1,500	19.71	0.80
LSD	NS	0.06
	60 days after transplanting	
Control	19.23	0.43
1,500	19.24	0.74
LSD	NS	0.07

[z] Means separation within columns of each growth stage by least significant difference (LSD) test at $P = 0.05$

changes in chlorophyll a and b in strawberries.

The anthocyanin content varied with the growth stages. The anthocyanin content was lowest at 0.1 mg/100g, but it increased as the growth days progressed after transplantation.

The 'Yeolpungjeokchima' cultivar used in this experiment requires a certain period for the leaves to express red coloration. In the early growth stage after transplanting, when only about two leaves have unfolded, since it is a phase of vigorous growth, anthocyanin expression did not occur.

However, until 45 days after transplantation, both the CO_2 treated group and the control group showed an increase in anthocyanin content. The CO_2 treated group had higher anthocyanin content than the control group throughout all growth periods, and on the 60th day after transplantation, the anthocyanin content was 0.74 mg/100g in the CO_2 treated group and 0.43 mg/100g in the control group. These results are consistent with previous studies (Park et al., 2012), indicating that high concentrations of CO_2 supply increase anthocyanin content in lettuce.

The results of investigating water soluble vitamins when supplying CO_2 for lettuce cultivation are presented in Table 4. Water soluble vitamins including vitamin C, vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B3 (niacin), vitamin B5 (pantothenic acid), and vitamin B6 (pyridoxine) were examined.

On the 60th day after transplanting, the content of all the water soluble vitamins except B3 in lettuce of the CO_2 treatment group showed slightly higher or similar results compared to the control group (Table 4). Particularly for B3, it was 1.5 mg/kg in the CO_2 treatment group,

Table 4. Quantification of six types of water soluble vitamins in 'Yeolpung jeokchima' lettuce

CO_2 supply (ppm)	Vitamin C	Vitamin B1	Vitamin B2	Vitamin B3	Vitamin B5	Vitamin B6
	Concentration (mg/kg)					
Control	3.2±0.3[z]	0.1±0.001	1.9±0.13	0.9±0.05	2.3±0.04	0.1±0.01
1,500	3.3±0.2	0.1±0.01	1.8±0.23	1.4±0.07	2.4±0.04	0.1±0.02

[z] Standard deviation (SD)

Table 5. Effect of CO_2 supply treatment on macro nutrient for 60 days after transplanting of 'Yeolpung jeokchima' lettuce at 20℃ in greenhouse

CO_2 supply (ppm)	T-N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Mn (mg/kg)	Zn (mg/kg)
Control	4.98	4.20	5.62	1.22	0.88	217.8	101.0
1,500	5.13	4.46	3.93	1.36	0.98	234.1	102.0

which was 0.5 mg higher than that in the control group.

However, Zhu et al.(2018) reported that high CO_2 concentration reduces the content of water soluble vitamins such as B1, B2, B5, and B9 in rice. Additionally, Taub et al.(2008) found that high CO_2 levels decrease protein content in crops such as rice, wheat, beans, and potatoes, suggesting that a decrease in plant nitrogen content can also affect the content of vitamins. Therefore, it can be inferred that the contents of water soluble vitamins may vary depending on the levels of the CO_2 treatments and the crop cultivars. However, in this study, there was a tendency for the content of water-soluble vitamins to slightly increase due to CO_2 supplement.

CO_2 supplement in lettuce cultivation was investigated for its effect on the mineral content of plants (Table 5). In most cases, except for potassium (K), the majority of the macro nutrients were higher in the CO_2 treatment group compared to the control group. The total nitrogen (T-N) content which represents overall nitrogen levels, was slightly higher in the CO_2 treatment group at 5.13% compared to the control group at 4.98%. However, potassium (K) content was higher in the control group at 5.62% compared to 3.93% in the treatment group.

Combining this study and the result of the previous research (Fangmeier et al., 1997), demonstrating the inorganic componets such as K, Mn, P, and Mg increases, it can be concluded that the inorganic componets like macro nutrients in the plant body increase even with the supplement of CO_2 in the atmosphere.

4. Conclusion

Research on the effects of CO_2 usage in greenhouse lettuce cultivation is lacking, and there is an urgent need for such studies. This study was conducted to investigate the effects of CO_2 supplement on growth and quality in greenhouse lettuce cultivation. When CO_2 was supplied at 1,500 ppm in lettuce cultivation, overall growth parameters such as number of leaves, leaf area, plant length, fresh weight, and dry weight were superior compared to the control group. While there was no significant difference in relative growth rate due to CO_2 supplement, an increase in leaf area index was observed with CO_2 usage.

Furthermore, although there was no significant difference in the content of water-soluble vitamins such as Vitamin C, B1, B2, B5, and B6 due to CO_2 supplement, the Vitamin B3 content in the CO_2 treatment group was 0.5 mg/kg higher than in the control group. Additionally, the inorganic content including P, Ca, Mg, Mn, and Zn, as well as the total nitrogen content ratio (T-N ratio), were higher in the CO_2 treatment group.

Therefore, the use of CO_2 in lettuce cultivation resulted in increased yield and promoted growth, enabling early harvesting. These findings provide scientific evidence that can be applied to enhance both the growth and quality of lettuce cultivation.

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