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Effects of CO_2 Enrichment on Carbon Assimilation, Yield and Quality of Oriental Melon Cultivated in a Solar Greenhouse

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Abstract: Since CO_2 is the fundamental substrate for photosynthesis, fluctuating concentrations have a direct effect on plant growth and metabolism. Accordingly, CO_2 enrichment within a certain range was found to improve photosynthesis, yields and the quality of plants. In order to further understand the underlying impact of CO_2 enrichment, this study employed an open-top chamber growth box model with the following two treatments: control treatment (CO_2 concentration: 380 ± 30 μL/L) and CO_2 enrichment (1200 ± 50 μL/L). The effects on leaf carbon assimilation, fruit yield and quality were subsequently determined. The net photosynthetic rate, intercellular CO_2 concentration, dry matter accumulation and soluble sugar content in the oriental melon leaves increased significantly on day 5 of CO_2 enrichment. Moreover, a significant increase in the activity of carbon assimilation-related enzymes Rubisco, RCA, FBPase and CA was also observed, with the upregulation of *CmRubisco*, *CmRCA*, *CmFBPase* and *CmCA* gene expression from day 15 of CO_2 enrichment. Thus, the yield per plant and content of soluble sugars and soluble solids in the fruit also increased significantly. These findings suggest that CO_2 enrichment has positive effects on oriental melon growth, increasing photosynthesis and the activity of photosynthetic carbon-assimilation-related enzymes and associated gene expression, thereby improving fruit yields and quality. These results provide a foundation for the CO_2 enrichment of oriental melon cultivated in solar greenhouses in autumn/winter and winter/spring.

Keywords: CO_2 enrichment; oriental melon; carbon assimilation; yield and quality

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1. Introduction

A solar greenhouse is an important facility for winter vegetable cultivation in Northern China [1]. In recent years, the cultivation area of winter/spring and autumn/winter crops in these facilities is increasing, with the frequent use of closed heat preservation and plastic film mulching to counteract the low outdoor temperatures. Therefore, CO_2 concentrations within the greenhouse decrease rapidly and cannot meet the needs of photosynthesis, affecting both the yield and quality of the crop [2]. The oriental melon (*Cucumis melo* var. *makuwa* Makino) belongs to Cucurbitaceae family and is an annual vegetable crop that is native to China, Japan and India. The fruit is used as the edible organ, and the growth period is approximately 90 days. It is generally cultivated in solar greenhouses in winter in Northern China; however, the lack of CO_2 limits production during winter/spring and autumn/winter. To counteract this, studies suggest that CO_2 enrichment can promote photosynthesis and water use efficiency, enhancing plant growth [3–5]. Under suitable cultivation conditions, CO_2 fixation can affect the photosynthetic capacity of plants [6]. Previous experimental studies found that CO_2 enrichment can promote plants to capture light energy and CO_2 fixation, improving the photosynthetic rate, and thus, enhancing

the photosynthetic capacity of the plant [7–9]. For example, the net photosynthetic rate of C_3 plants exposed to high concentrations of CO_2 for a short period of time was found to increase by 10%–50% [10]. CO_2 enrichment was also found to promote the growth vigor of tomato seedlings, increasing productivity by 19.32% [11,12]. In addition, CO_2 enrichment was also found to increase organic matter synthesis, promoting yields [13], while increases in the fruit setting rate and accelerated fruit expansion were also observed, significantly promoting the early and total tomato yields [14]. An overall improvement in the quality of tomato fruit was also observed under CO_2 enrichment [15,16], with significant increases in nutritional and sensory qualities, both of which are conducive to fruit enlargement and the deepening of fruit color during tomato cultivation in solar greenhouses [17,18]. Therefore, CO_2 enrichment is an important basic technology to increase crop yields and economic benefits in the solar greenhouse [19].

Photosynthetic carbon assimilation refers to the process whereby plants use chemical energy (ATP and NADPH) formed by photosynthesis and photoreactions to produce carbohydrates and other organic substances, through a series of electron transfer and enzymatic reactions during the dark reaction of photosynthesis. Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is a key enzyme in plant carbon assimilation, catalyzing the first step of CO_2 fixation during photosynthetic carbon assimilation, as well as the first step of photorespiration. Rubisco also plays a crucial role in balancing the metabolic ratio of plant photosynthesis and photorespiration. In C_3 plants, the Rubisco enzyme is the main limiting factor of photosynthetic CO_2 assimilation, the activity of which is affected by various factors [20,21]. Accordingly, the activity of the Rubisco enzyme in the leaves was found to be linearly correlated with the net photosynthetic rate [22]. Rubisco activating enzyme (RCA) regulates the initial carboxylation activity and degree of activation, as well as the carboxylation efficiency of Rubisco [23]. The degree of activation of Rubisco determines the carbon assimilation efficiency of the leaves and is a key factor in carbon assimilation [24]. Fructose-1,6-bisphosphatase (FBPase) is one of the key enzymes regulating the Calvin cycle, with activity directly affecting the rate of photosynthesis and carbohydrate accumulation [25]. Carbonic anhydrase (CA) catalyzes the reversible hydration of CO_2 and is also closely related to photosynthesis [26], with its activity playing a key role in photosynthesis, crop yields and quality [27–29]. Meanwhile, as the fundamental substrate for the CA catalytic reaction, CO_2 affects the subsequent concentration. For example, at low concentrations of CO_2, CA activity is high, while, at high concentrations, CA activity is low, thus maintaining the relative stability of photosynthesis [30]. The effect of CO_2 enrichment on plant growth and physiology is therefore realized by the rate of CO_2 fixation and the expression of genes related to chlorophyll metabolism [31]. In line with this, GO enrichment analysis revealed that CO_2 enrichment resulted in the significant enrichment of the genes related to thylakoids and photosynthesis [32].

As the essential carbon source, CO_2 has an important impact on photosynthesis [33]. However, research into the effect of CO_2 enrichment on photosynthesis under greenhouse cultivation tends to focus on the effects of factors related to photosynthesis, rather than the underlying mechanisms of internal carbon assimilation. In this study, the oriental melon was used to examine the effect of CO_2 enrichment under solar greenhouse cultivation during the autumn/winter and winter/spring. The effects on carbon assimilation, related enzymes and gene expression and fruit yields and quality were subsequently examined. The findings provide a foundation for the management of oriental melon yields and quality under protected cultivation, as well as an important reference for further studies into photosynthetic performance and carbon assimilation.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

The oriental melon cultivar 'Yumeiren' was selected as the experimental material. Plants were grown in the Liaoshen I solar greenhouse, Shenyang, Liaoning Province, China, from March to June 2021.

Cultivation was based on the open-top chamber (OTC) growth box model. The growth boxes consisted of two small vaulted sheds covered in polyethylene droplet-free film, with length × width × height dimensions of 5 × 0.8 × 1.8 m (Figure 1). A CO_2 ventilation pipe was attached to the vaulted roof of the shed in the shape of a "mouth". The pipe was then pierced with 1-mm-diameter holes every 10 cm to allow the release of CO_2. The height of the ventilation pipes was adjustable as necessary. A small fan was then placed in each corner of the bottom of the vaulted shed to ensure a uniform CO_2 concentration. A cylinder of liquid CO_2 was used as the carbon source, and the CO_2 concentration was regulated using a CO_2 monitoring system (GMM220, Vaisala, Vantaa, Finland). To ensure that the temperature within the vaulted shed remained within the appropriate range for plant growth, the plastic film on both sides and the top of the shed was removed where necessary and then reattached during treatment. This method allowed for ventilation, cooling and daily management when the midday temperatures became too high. Such devices were previously tested and used in tomato [34], cucumber [35] and other crops. The solar radiation value was varied between 400 and 700 $W \cdot m^{-2}$ and the oxygen content was 21% during the treatment in the solar greenhouse. The ventilation of the solar greenhouse was controlled by an automatic ventilator, and the temperature was adjusted according to the growth of seasonal crops (on sunny days with high temperatures, ventilation should be carried out 2–3 times a day for 15–30 min each time). The open air temperature of the greenhouse was 28 °C, and the closed air temperature was 24 °C.

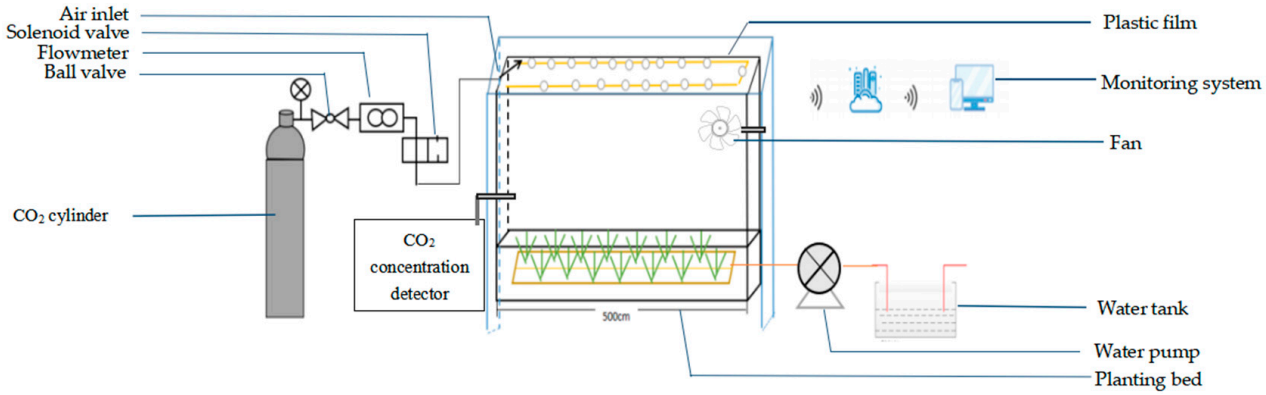

Figure 1. The CO_2 enrichment system based on the open-top chamber method.

When the seedlings grew to three-leaf stage, vigorous seedlings showing similar growth potential were transplanted to 17-cm-diameter pots and placed in different compartments, with a total of 90 plants per compartment. Plants were spaced at a distance of 25 cm, with 40 cm between rows. Two CO_2 concentrations were then applied as follows: 1200 ± 50 μL/L (CO_2 enrichment group, S1) and 380 ± 30 μL/L (control group, CK). Treatment was carried out between 9:00 and 11:30 on a sunny day, for approximately 2.5 h per day, but not on rainy or snowy days, or when the film was opened for ventilation. Physiological indexes were measured at 0, 5, 10, 15, 20 and 25 days after treatment in functional leaves (3rd and 4th segments from the top) from both the control and treatment groups. The leaves were wrapped in foil, immediately frozen in liquid nitrogen and then stored in a refrigerator at −80 °C until use. Fruit-related indexes were also measured at the fruit stage.

2.2. Environmental Variables within the Greenhouse

Figure 2A shows the daily changes in CO_2 concentrations within the solar greenhouse. Data were recorded using a CO_2 intelligent control system across 3 days under normal weather, and then the average value was calculated. The concentration of CO_2 in the greenhouse was generally low between 9:00 and 11:30. The reason for this is thought to be increased light intensity in the solar greenhouse, and enhanced plant photosynthesis,

resulting in a rapid reduction in the CO_2 concentration. The CO_2 monitoring system was placed in the vaulted shed according to the OTC model to directly monitor the supply of air from the carbon dioxide cylinder. Meanwhile, under the S1 treatment, the CO_2 concentration was maintained at 1200 μL/L. Changes in the average CO_2 concentration, temperature and relative humidity within the greenhouse were subsequently measured between 9:00 and 11:30 from 9 May to 2 June 2021 (Figure 2B–D, respectively), as well as daily variations in light intensity (Figure 2E).

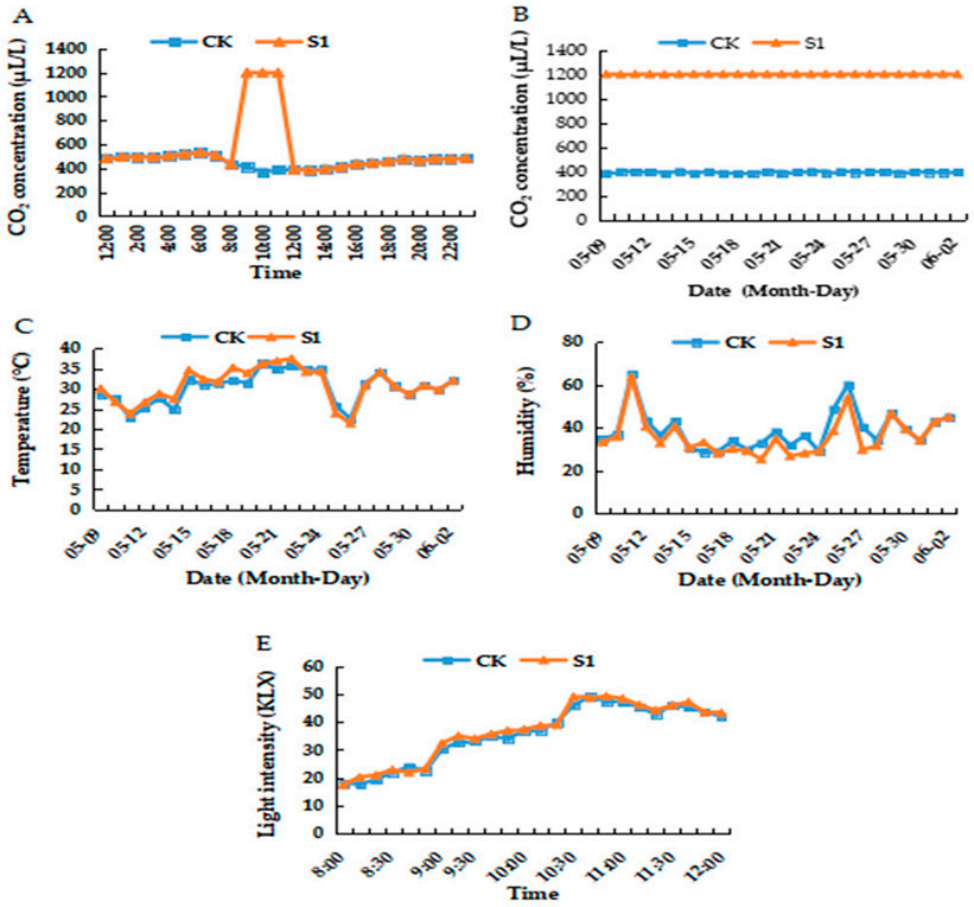

Figure 2. Changes in internal environment in solar greenhouse. Note: (**A**,**E**) Daily variation in CO_2 concentration and light intensity in treatment group (S1) and control group (CK) on the day of treatment. (**B**–**D**) Changes in average CO_2 concentration, average temperature and relative humidity in the space of control group (CK) and treatment group (S1) between 9:00 and 11:30 on the day of treatment.

2.3. Measurement Items and Methods

2.3.1. Measurement of Growth Indexes

Plant height was measured using a tape measure, and stem diameter was determined using vernier calipers (Mitutoyo, Schweiz, AG, Urdorf, Germany). The plant samples were separated into root, stem and leaf samples for the measurement of above- and belowground fresh weights using a 1/10,000 balance. They were then dried and weighed to determine the dry weights.

2.3.2. Measurement of Photosynthetic Parameters and Photosynthetic Pigments

Photosynthesis in oriental melon leaves was determined using a Li-6800 photosynthesis system (LI-COR, Lincoln, NE, USA) between 9:00 and 11:30 a.m. on a sunny day. The following parameters were recorded: the net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO_2 concentration (Ci) and transpiration rate (Tr) under saturated

light. The content of photosynthetic pigments was then determined as follows: 0.1 g leaves were extracted with acetone and ethanol (1:1) and analyzed by a UV–vis spectrophotometer (Beijing LabTech Instruments Co., Ltd., Beijing, China).

2.3.3. Measurement of Soluble Sugar in the Leaves

The total soluble sugar content of the leaves was determined using anthrone colorimetric method, while the content of glucose, fructose and sucrose was determined using high-performance liquid chromatography (HPLC) [36]. The leaf samples (1 g) were extracted with 80% ethanol and soaked in 80 °C water for 1 h, and this was repeated 3 times. After a constant volume concentration was achieved, they were dissolved in 1 mL ultra-pure water. An amino acid column was used. The supernatant was treated with 0.45 μm and 0.22 μm filtration membranes and evaluated by a Waters 600E device (Waters, Milford, CT, USA). The moving phase was 75% acetonitrile and 25% ultra-pure water. Waters Millennium was used for control and data processing.

2.3.4. Measurement of Fruit-Related Indexes

Fruit weight and yield per plant were determined using a balance with accuracy of one percent. The skin was removed and the flesh was sampled for the content of total soluble sugars, soluble protein, vitamin C and organic acids based on the anthrone colorimetric method [37], Coomassie bright blue G-250 method [38], molybdenum blue colorimetric method [39] and NaOH titration method [40], respectively. Determination of soluble solids was achieved with a hand-held refractometer (China Opto-Electro Industries Co., Ltd., Beijing, China).

2.3.5. Measurement of Carbon-Assimilation-Related Enzyme Activity and Gene Expression

In this study, Rubisco, RCA, FBPase and CA were selected to analyze the effects of CO_2 enrichment on the key enzyme activity of carbon assimilation in oriental melon leaves. The activity of the four enzymes was determined according to the instructions in the ELISA kit (Jiangsu Boshen Biotechnology Co., Ltd., Nanjing, China). For the analysis of gene expression, total RNA was extracted from the leaves using an Ultrapure RNA kit (Beijing Kangwei Century Biotechnology Co., Ltd., Beijing, China). The RNA was then reverse-transcribed into cDNA using a reverse transcription kit (Monad Biotechnology Co., Ltd., Suzhou, China). Quantitative gene expression analysis was performed using the method of operation of a fluorescence quantitative kit (DRR04A, TANGEN) in a Jena real-time fluorescence quantitative analyzer. Fluorescence quantification was carried out under 45 cycles of 95 °C for 30 s, 95 °C for 5 s, 60 °C for 34 s and 60 °C for 15 s. The four key genes in carbon assimilation, *CmRubisco*, *CmRCA*, *CmFBPase* and *CmCA*, were selected as target genes, with actin as an internal reference. The gene login numbers and primer sequences are shown in Table 1. The $2^{-\Delta\Delta CT}$ method was used to calculate the relative gene expression.

Table 1. Specific primers for qRT-PCR.

Gene	Primer Sequences 5′-3′	Accession Number
Actin	(F)AAGGCAAACAGGGAGAAGATGA (R)AGCAAGGTCGAGACGTAGGATA	
CmRCA	(F)CAACGATGTGGAGGGTTTTTAC (R)TATGTCTGCTGCTTCACGGTAC	MELO3C008231.2
CmCA	(F)CCTCTATTTTCGCTTTCTCTCTTT (R)TTTCAGGTCCATGTGAACCCT	MELO3C009476.2
CmFBPase	(F)TCTCGTCGCTTCTCCCTTCA (R)GCCATCACAGCAACTTTTCCA	MELO3C018610.2
CmRubisco	(F)TCGCAAGAACAACGACATCAC (R)TCACGGTAAACGAATCCACTG	MELO3C012252.2

F: Forward. R: Reverse.

2.4. Statistical Analysis

Microsoft Excel 2013 (Microsoft, Redmond, WA, USA) was used for data analysis and mapping, SPSS 22.0 (IBM, Armonk, NY, USA) was used for one-way analysis of variance (ANOVA), and Tukey's post-hoc test was used for the analysis of significance at $p < 0.05$. All data are expressed as the mean ± standard error (SE) of three independent biological repeats per treatment.

3. Results

3.1. Effects of CO_2 Enrichment on Growth of the Oriental Melon Plants in the Solar Greenhouse

Observation and analysis of plant height for 25 consecutive days under CO_2 enrichment revealed accelerated growth of the oriental melon plants. As shown in Figure 3, plant height was significantly greater under the S1 treatment compared to the CK from day 5 until the end of treatment. On day 15, the difference between S1 and CK was the greatest, with an increase in height of 50%. Meanwhile, the stem diameter showed a rapid followed by a more gradual increase under the S1 treatment, and was significantly greater than in the CK. On day 15 of treatment, the stem diameter increased significantly by 17.1% under the S1 treatment compared with the CK. Overall, therefore, CO_2 enrichment promoted the growth of oriental melon.

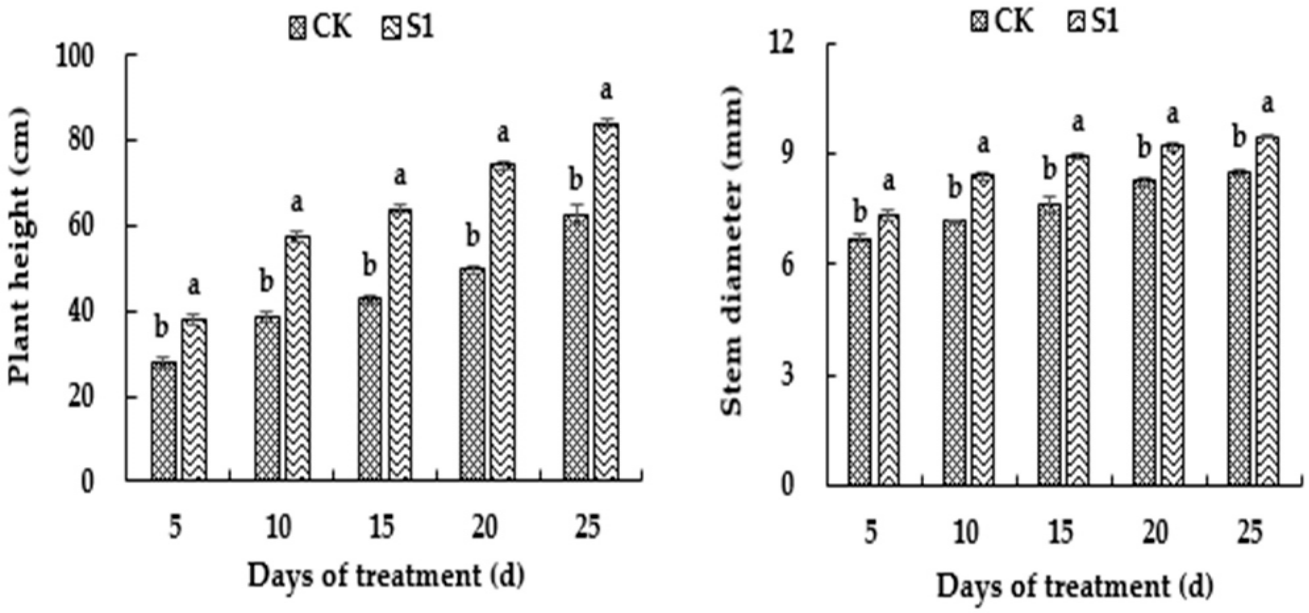

Figure 3. Effects of CO_2 enrichment on growth of the oriental melon plants in the solar greenhouse. Error bars ($n = 3$) indicate the standard error (SE), and different lowercase letters indicate a significant difference between treatments ($p < 0.05$).

3.2. Effects of CO_2 Enrichment on Biomass of the Oriental Melon Plants in the Solar Greenhouse

To a certain extent, the plant biomass reflects the strength of plant growth and photosynthetic capacity. As shown in Figure 4, CO_2 enrichment resulted in a significantly higher biomass for both the above- and belowground parts compared to the CK. From day 5 of treatment, all biomass indexes were significantly greater under the S1 treatment, with the most significant difference on day 20. Compared with the CK, the S1 treatment resulted in increases in the aboveground fresh and dry weights of 53% and 45.96%, and the belowground fresh and dry weights of 34.17% and 40.52%, respectively. Therefore, CO_2 enrichment resulted in a significant increase in the oriental melon's biomass.

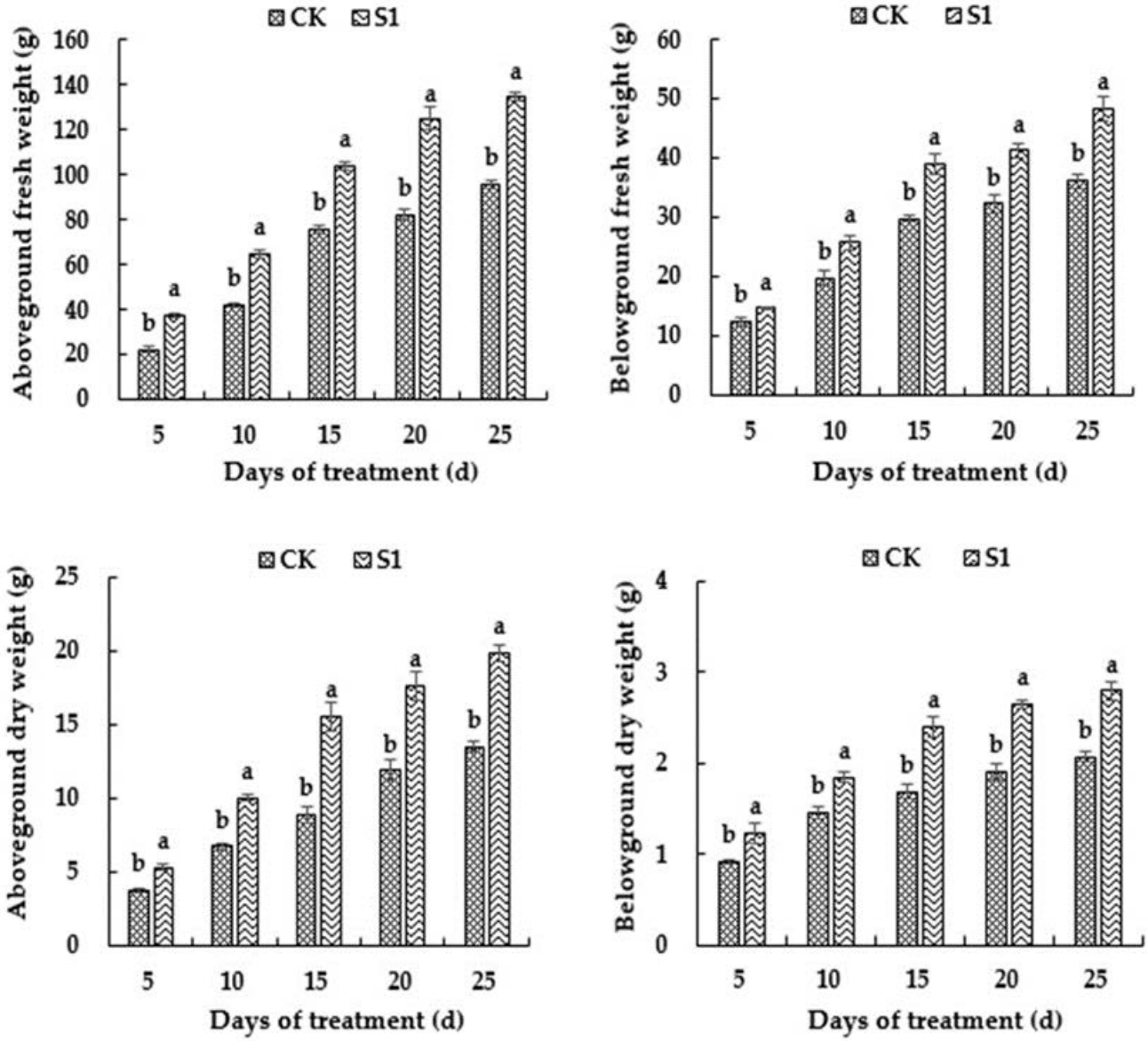

Figure 4. Effects of CO_2 enrichment on aboveground and belowground biomass of the oriental melon plants in the solar greenhouse. Error bars (n = 3) indicate the standard error (SE), and different lowercase letters indicate a significant difference between treatments ($p < 0.05$).

3.3. Effects of CO_2 Enrichment on Photosynthetic Pigment Content of the Oriental Melon Leaves in the Solar Greenhouse

Photosynthetic pigments allow green plants to absorb light energy and synthesize CO_2 and water into organic matter. As shown in Figure 5, CO_2 enrichment in the solar greenhouse was conducive to the increased accumulation of photosynthetic pigments in the leaves. During the S1 treatment, chlorophyll *a* and chlorophyll *b* increased on day 5, but not significantly. There was a temporary decrease after 10 days of treatment. On day 20 of treatment, the chlorophyll *a* and chlorophyll *b* content reached a peak, increasing significantly by 15.3% and 16.67% compared with the CK, respectively. These results suggest that CO_2 enrichment promotes the absorption of light energy and subsequent photosynthesis in the oriental melon leaves.

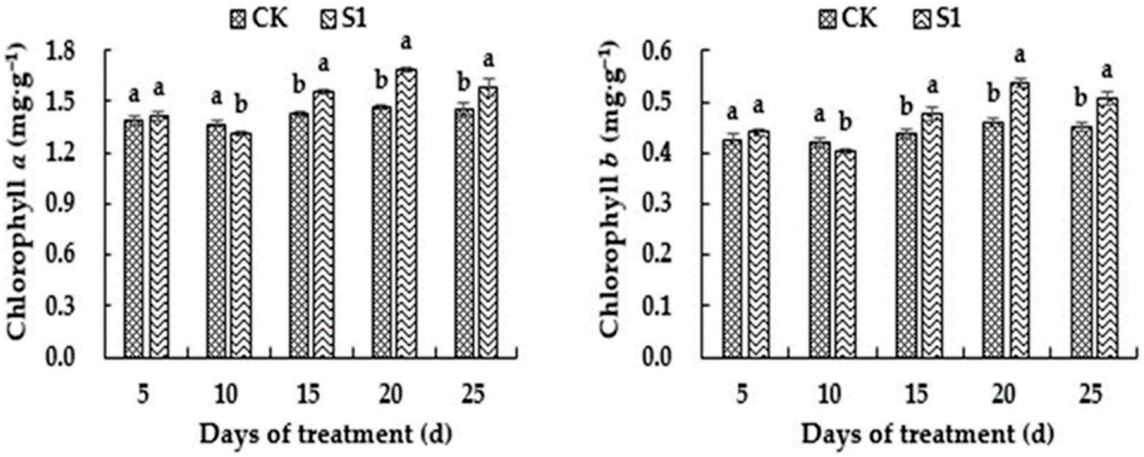

Figure 5. Effects of CO_2 enrichment on the chlorophyll content of the oriental melon leaves in the solar greenhouse. Error bars (n = 3) indicate the standard error (SE), and different lowercase letters indicate a significant difference between treatments ($p < 0.05$).

3.4. Effects of CO_2 Enrichment on Photosynthetic Parameters of the Oriental Melon Leaves in the Solar Greenhouse

Photosynthetic parameters are important indicators of plant photosynthesis. Figure 6 shows the changes in photosynthetic parameters in the oriental melon leaves under CO_2 enrichment. The net photosynthetic rate showed a rapid followed by a more gradual increase, reaching a peak on day 20, with a significant increase of 22.5% compared with the CK. Meanwhile, stomatal conductance and the transpiration rate were significantly lower under S1 from days 10 to 25 of treatment. Stomatal conductance was reduced significantly by 15.56% on day 20, while the transpiration rate showed a rapid followed by a slower increase. The transpiration rate was significantly inhibited under S1, and the difference was most significant on day 25 of treatment, with a reduction of 22.09% compared with the CK. Meanwhile, the overall intercellular CO_2 concentration tended to decrease and then increase under the S1 treatment, and was significantly higher than in the CK. The most significant difference was observed on day 20, with an increase of 133.73%. Overall, therefore, CO_2 enrichment promoted photosynthesis in the oriental melon leaves. The net photosynthesis rate is a physiological index reflecting photosynthetic carbon assimilation: the greater the rate, the stronger the carbon assimilation capacity. CO_2 enrichment therefore promoted carbon light assimilation in the oriental melon leaves.

3.5. Effects of CO_2 Enrichment on the Soluble Sugar Content of the Oriental Melon Leaves in the Solar Greenhouse

The soluble sugar content reflects the level of carbohydrate accumulation, and, therefore, the strength of plant vitality. As shown in Figure 7, CO_2 enrichment had differing effects on the content of different types of soluble sugars in the oriental melon leaves. Under the S1 treatment, the content of total soluble sugars showed a rapid followed by a more gradual increase, and was significantly higher compared to the CK treatment. Meanwhile, the fructose content was significantly lower under the S1 treatment on day 5, but increased significantly from days 10 to 25, reaching a peak on day 25. The sucrose content was also significantly lower under the S1 treatment on day 5, but increased rapidly from days 5 to 10, and remained significantly higher than in the CK until the end of treatment. On day 5 of treatment, the glucose content was significantly lower under the S1 treatment, but a rapid, significant increase was observed from days 5 to 10 compared with the CK. Therefore, CO_2 enrichment had a strong effect on the sucrose content and a significant impact on the content of other types of soluble sugars in the oriental melon leaves. In conclusion, CO_2 enrichment promotes the synthesis and accumulation of sugars in the oriental melon leaves.

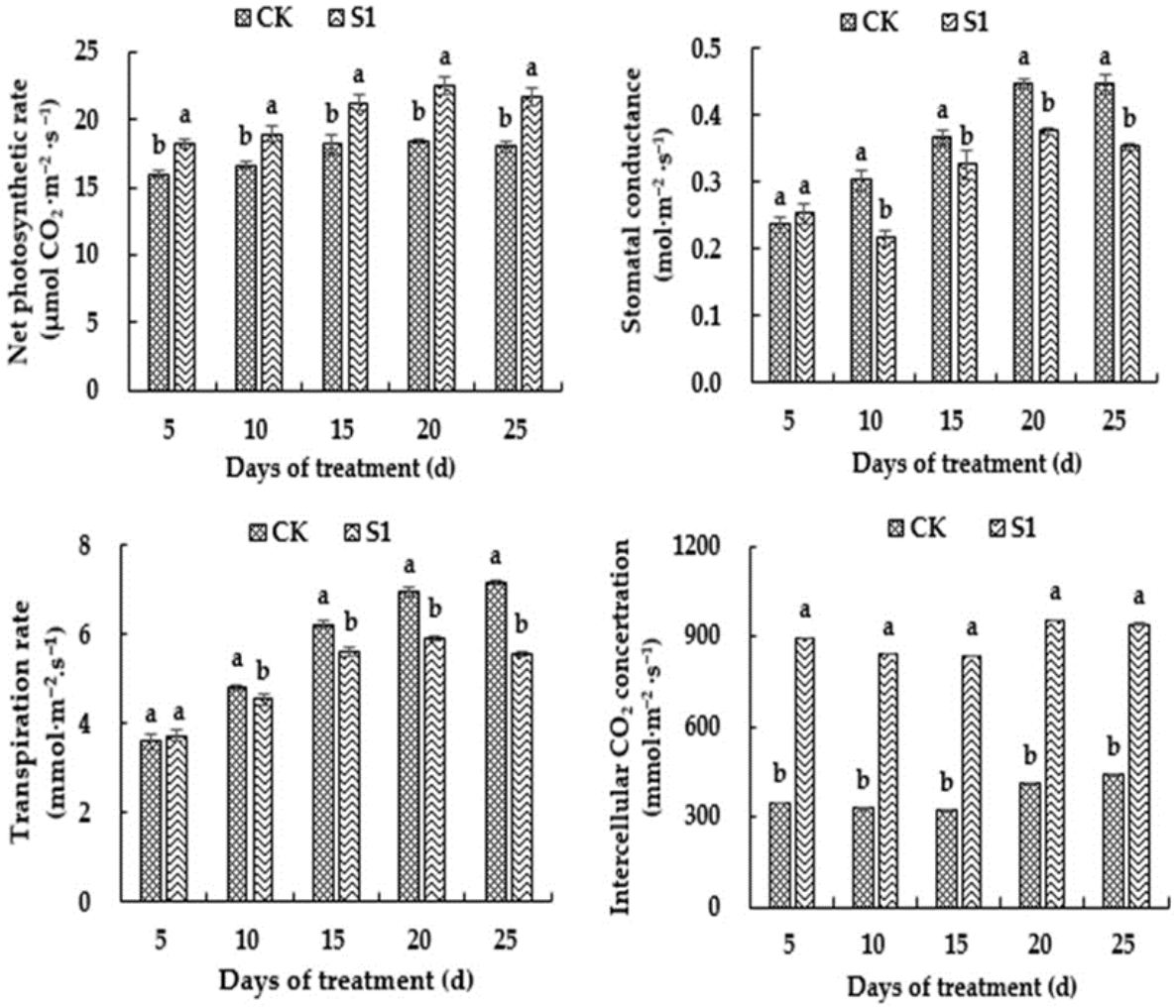

Figure 6. Effects of CO_2 enrichment on photosynthetic parameters of the oriental melon leaves in the solar greenhouse. Error bars (n = 3) indicate the standard error (SE), and different lowercase letters indicate a significant difference between treatments ($p < 0.05$).

3.6. Effects of CO_2 Enrichment on Activity of Carbon-Assimilation-Related Enzymes of the Oriental Melon Leaves in the Solar Greenhouse

During photosynthesis, Rubisco, RCA, FBPase and CA in the leaves act as light-regulating enzymes. As shown in Figure 8, CO_2 enrichment promoted the activity of all four enzymes, with a significant positive correlation with the CO_2 concentration. On day 5 of treatment, Rubisco activity was significantly lower under the S1 treatment compared to the CK; however, from day 10, a significant increase was observed. Meanwhile, RCA activity showed an overall increase with increasing treatment time, and was higher under the S1 treatment, except on day 10, when activity was significantly higher under the CK treatment. FBPase activity was also significantly higher under the S1 treatment on day 5, but was significantly lower than in the CK on day 10. On day 15, no significant difference was observed between treatments; however, the activity increased significantly with increasing treatment time under the S1 treatment compared to the CK. Overall, CA enzyme activity was higher under the S1 treatment than that of CK, with a more significant difference in the early stages of treatment. On day 5, CA activity was significantly higher by 55.58%; however, from day 15, the difference between treatments decreased gradually until day 25 of treatment. On day 25, there was no significant difference. These findings suggest that CO_2 enrichment significantly increased the activity of the above four enzymes, promoting carbon assimilation and thus the rate of photosynthesis in the oriental melon leaves.

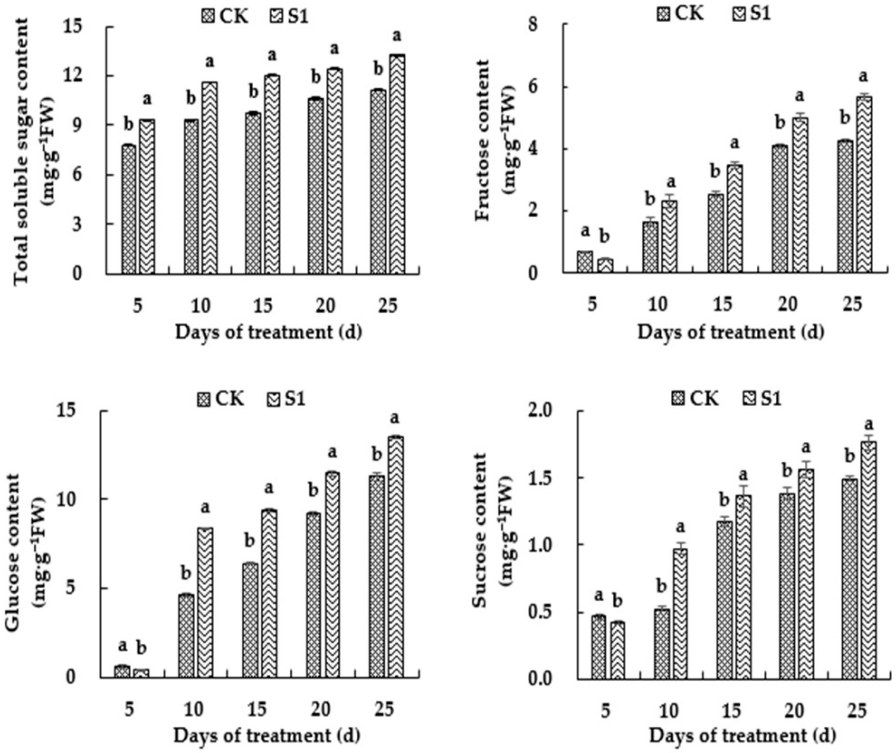

Figure 7. Effects of CO_2 enrichment on the soluble sugar content of the oriental melon leaves in the solar greenhouse. Error bars (n = 3) indicate the standard error (SE), and different lowercase letters indicate a significant difference between treatments ($p < 0.05$).

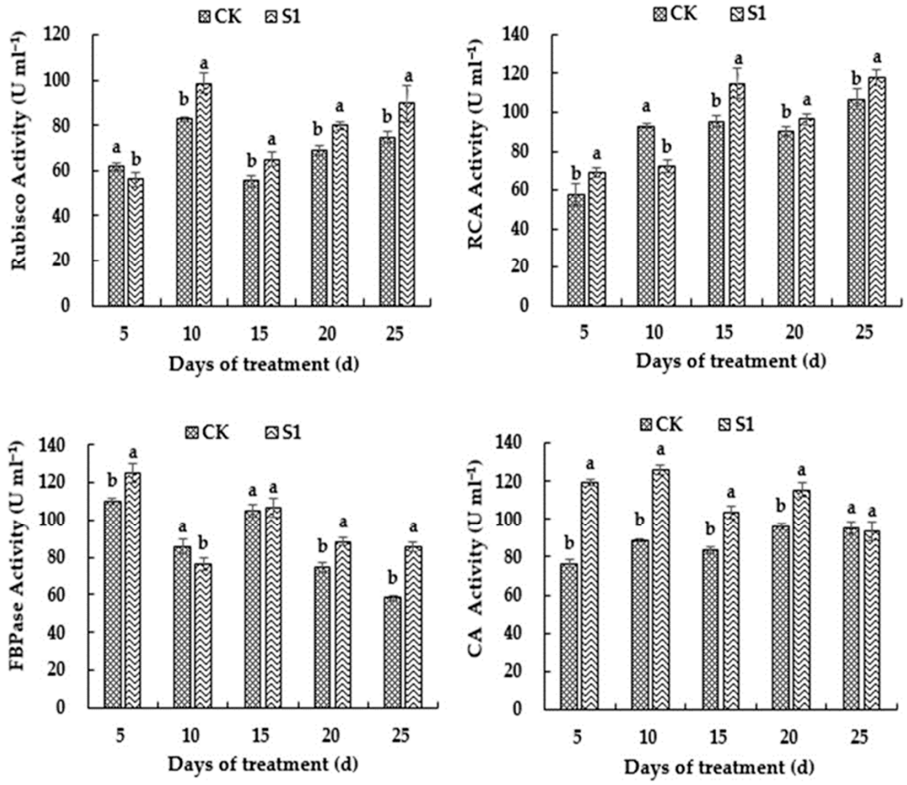

Figure 8. Effects of CO_2 enrichment on the activity of carbon-assimilation-related enzymes of the oriental melon leaves in the solar greenhouse. Error bars (n = 3) indicate the standard error (SE), and different lowercase letters indicate a significant difference between treatments ($p < 0.05$).

3.7. Effects of CO_2 Enrichment on Gene Expression of Carbon-Assimilation-Related Enzymes of the Oriental Melon Leaves in the Solar Greenhouse

The process of photosynthetic carbon assimilation in plants is jointly regulated by a variety of enzymes, the activity of which is affected by related gene expression. As shown in Figure 9, the key genes of carbon assimilation were significantly affected by the CO_2 concentration. *CmRubisco*, which regulates photosynthesis, was significantly different between the S1 and CK treatments. On day 5 of treatment, expression was significantly higher under the S1 treatment, while, on day 25, expression was significantly higher under CK. Meanwhile, the expression of *CmRCA*, which determines the activity of Rubisco, was significantly lower under S1 than the CK treatment from days 5 to 10, but significantly higher under the S1 treatment from days 15 to 25. The expression of *CmFBPase*, which determines the distribution of carbon elements to final products, was also significantly different between treatments from day 10. Between days 10 and 20, activity was significantly higher under the S1 treatment, while, on day 25, expression was significantly lower compared with CK. The expression of *CmCA*, which is closely related to photosynthesis, fluctuated with the treatment time. On day 5, expression was significantly higher under the S1 treatment, while, on day 10, expression was lower compared with CK. On days 15–25, expression was again higher under the S1 treatment compared with CK. Overall, the relative expression of *CmRubisco, CmFBPase, CmRCA* and *CmCA* increased by 31.6%, 38.9%, 26.8% and 27.7% with increasing CO_2, respectively. These results suggest that the gene expression of carbon-assimilation-related enzymes was significantly upregulated following 15 days of CO_2 enrichment.

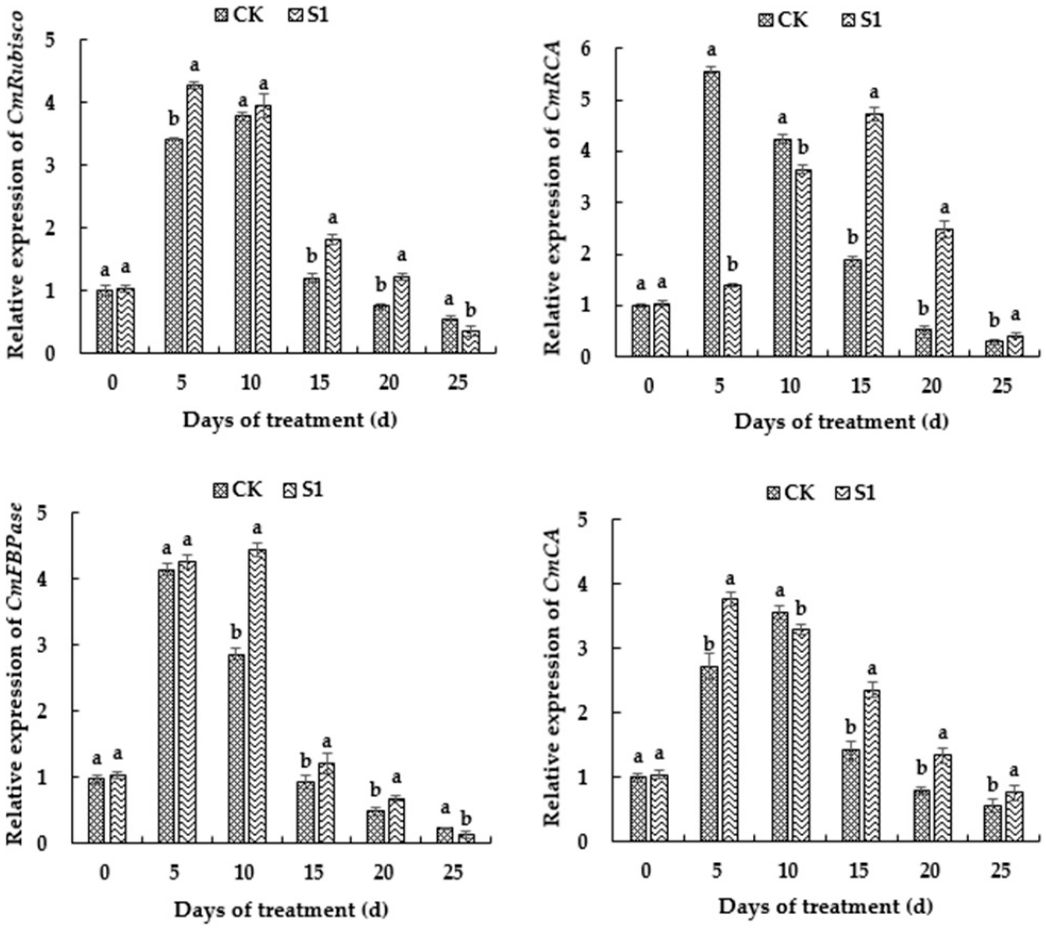

Figure 9. Effects of CO_2 enrichment on gene expression of carbon-assimilation-related enzymes of the oriental melon leaves in the solar greenhouse. Error bars (n = 3) indicate the standard error (SE), and different lowercase letters indicate a significant difference between treatments ($p < 0.05$).

3.8. Effects of CO_2 Enrichment on Yield and Quality of the Oriental Melon Fruit in the Solar Greenhouse

Table 2 shows the effect of CO_2 enrichment on the yield and quality of oriental melon fruit in the solar greenhouse. Compared with the CK treatment, the single fruit weight and yield under the S1 treatment increased by 29.91% and 10.86%, respectively. Moreover, the content of soluble solids, vitamin C, soluble protein and soluble sugars increased significantly by 25.91%, 9.09%, 26.81% and 43.09%, respectively, compared with CK. The overall quality also increased significantly, with an increase in the organic acid content of 44.44% and an increase in the sugar acid ratio of 33.1% compared with CK. These findings suggest that CO_2 enrichment at the seedling stage had a significant impact on the yield and quality of oriental melon fruit.

Table 2. Effects of CO_2 enrichment on yield and quality of the oriental melon fruit in the solar greenhouse.

Treatment	Yield Per Plant (g)	Single Fruit Weight (g)	Soluble Solids (%)	Vitamin C (mg/g)	Soluble Protein (mg/g)	Soluble Sugars (%)	Organic Acids (%)	Sugar Acid Ratio
CK	1043 ± 6.08^{b}	496 ± 8.08^{b}	11.46 ± 0.15^{b}	25.72 ± 0.41^{b}	3.58 ± 0.04^{b}	12.46 ± 0.20^{b}	0.16 ± 0.05^{b}	68.23 ± 2.31^{b}
S1	1295 ± 7.63^{a}	638 ± 4.50^{a}	14.43 ± 0.20^{a}	28.06 ± 0.26^{a}	4.54 ± 0.12^{a}	17.83 ± 0.81^{a}	0.23 ± 0.03^{a}	90.82 ± 3.47^{a}

Note: CK: CO_2 concentration in greenhouse (380 ± 30 μL/L, control treatment); S1: CO_2 enrichment treatment (1200 ± 50 μL/L). Different letters in the same row indicate significance at 0.05 level among treatments.

4. Discussion

4.1. Effects of CO_2 Enrichment on Growth and Photosynthesis-Related Indexes of the Oriental Melon Plants in the Solar Greenhouse

Photosynthesis is the most basic plant reaction and the basis of plant growth. CO_2 comprises the fundamental substrate, directly affecting the photosynthetic rate, thus affecting plant growth and biomass accumulation [41,42]. In line with this, CO_2 enrichment was found to increase the fresh weight of lettuce by 29%–40% and the dry weight by 21%–40% [43]. Similarly, in this study, CO_2 enrichment significantly promoted plant height and stem diameter in an oriental melon from day 5 of treatment, with increases in the fresh and dry weights of both the aboveground and belowground parts compared with the CK group. These findings support the suggestion that CO_2 enrichment improves plant growth and development. A possible reason for this is that CO_2 enrichment increases the photosynthetic efficiency in the plant leaves, improving the accumulation of carbohydrates and the transport to each part of the plant. This then promotes the accumulation of organic matter, which is conducive to plant growth [44–47]. Under normal atmospheric CO_2 concentrations, oriental melon growth is relatively slow, possibly due to insufficient CO_2 for photosynthesis [48]. CO_2 enrichment in the solar greenhouse environment enhances the available CO_2, favoring growth and development. Similarly, a substantial increase in biomass accumulation and plant vitality was previously reported in tomato following CO_2 enrichment [49].

As a source of light energy absorption and transformation, chlorophyll comprises the basic substrate in primary photosynthesis reactions [50], providing energy, ATP and NADPH for photosynthetic carbon assimilation. In this study, CO_2 enrichment significantly increased the chlorophyll content of the oriental melon leaves, promoting photosynthesis. The reason may be that CO_2 enrichment causes the upregulated expression of genes that mediate chlorophyll synthesis, so its content increases [51], but the related genes were not studied in this work, so it remains to be further studied in the future. However, during the treatment period, the chlorophyll content temporarily decreased, which may have been caused by the fact that CO_2 enrichment promoted the synthesis of carbohydrates, accelerated the growth of leaves and reduced the nitrogen content in leaves, resulting in a reduction in the chlorophyll synthesis capacity [52]. The net photosynthetic rate (Pn) of the oriental melon leaves is one of the most important indicators of photosynthetic capacity, which was remarkably improved compared to the CK treatment throughout the study, indicating that under the conditions of CO_2 enrichment, the leaves can fix

more organic carbon, thus promoting the growth of oriental melon. Meanwhile, stomatal conductance and transpiration began to increase from day 5, albeit remaining significantly lower than in the CK from day 10 of treatment. This is thought to have been caused by the effect of the increase in CO_2 on the intercellular CO_2 concentration. In order to maintain the intercellular CO_2 partial pressure below that of the atmosphere, plants reduce intercellular CO_2 concentration by regulating the degree of stomatal opening and closing [35]. Plant guard cells sense the change in CO_2 and regulate the K^+ channels accordingly, depolarizing the cell membrane potential of the guard cells and reducing or closing the stomatal apertures, thereby reducing stomatal conductance [7]. CO_2 enrichment reduces the stomatal conductance and density, while resistance to the outward diffusion of water in the cells increases, causing a certain amount of water to be maintained within the cells [53–55], thus reducing the transpiration rate. In the short term, the photosynthetic rate increases with CO_2 enrichment, while, in the long term, this phenomenon gradually weakens with photosynthetic adaptation [56,57]. In this study, the photosynthetic rate remained higher than in the CK throughout the treatment in the solar greenhouse, possibly due to the fact that the CO_2 concentration used in this experiment was lower than the CO_2 saturation point of oriental melon leaves, or because this experiment was conducted in winter and spring at a time of low atmospheric temperatures, short day lengths and long nights. Photosynthetic products such as starch and soluble sugars accumulate in the leaves during the day, and are rapidly transferred or consumed by respiration at night, maintaining the balance between the source and sink and preventing the feedback inhibition of photosynthesis [58].

4.2. Effects of CO_2 Enrichment on Activity of Carbon-Assimilation-Related Enzymes and Associated Gene Expression of the Oriental Melon Leaves in the Solar Greenhouse

The Calvin cycle is the most basic method of photosynthetic carbon assimilation in plants. It is regulated on both the substrate level and the enzyme level. Rubisco catalysis is the first step of the Calvin cycle, while its role in photosynthesis is to fix CO_2, having a decisive effect on the net photosynthetic rate. Therefore, Rubisco is a key enzyme in photosynthesis, as well as a rate-limiting enzyme in the assimilation of CO_2. Moreover, abiotic stress is also known to affect the activity of Rubisco, reducing the Rubisco content [59]. In contrast, RCA activity has little direct influence on the photosynthetic rate; however, it plays an important role in regulating the initial activity of Rubisco, with the activation of Rubisco by RCA affects photosynthesis in plants [23]. FBPase is another important regulating enzyme in the Calvin cycle. Since FBPase contains a K^+ binding site, combination with K^+ increases its binding ability, thereby enhancing its activity and controlling the rate of photosynthesis [25]. Lastly, CA is involved in the morphogenesis of plant seedlings and in maintaining the photosynthetic capacity of the leaves [60]. Previous studies have confirmed the important role of Rubisco, RCA and FBPase in photosynthesis [61]. Meanwhile, the study on tomato revealed that carbon-assimilation-related enzymes have a certain effect on carbon assimilation [62], while, in this study, the activity of all four enzymes was found to increase significantly following 15 days of CO_2 enrichment. Therefore, CO_2 enrichment promotes the activity of carbon-assimilation-related enzymes, which speeds up the assimilation of carbon. In line with this, CO_2 enrichment also affects the expression levels of carbon-assimilation-related enzyme genes. In this study, the expression levels of *CmRubisco*, *CmRCA*, *CmFBPase* and *CmCA* were significantly higher under the S1 treatment compared to CK from day 15 of CO_2 enrichment in the solar greenhouse. On day 25 of treatment, the difference between the treatment and control decreased. This suggests that CO_2 enrichment increases the expression of key carbon-assimilation-related genes, enhancing the activity of carbon-assimilation-related enzymes and promoting photosynthetic carbon assimilation. The above results highlight the important effect of CO_2 enrichment on photosynthesis from the perspective of carbon assimilation. In addition, CO_2 concentrations affect the expression of carbon-assimilation-related genes, regulating the activity of associated enzymes and thereby the process of carbon assimilation.

4.3. Effects of CO_2 Enrichment on the Yield and Quality of the Oriental Melon in the Solar Greenhouse

Photosynthesis is also a decisive factor in the yield and quality of vegetables. As the main substrate for photosynthesis, CO_2 is a limiting factor for growth and development. CO_2 enrichment was therefore found to promote fruit growth and improve fruit quality, yield and resistance [63,64]. In line with this, the results of this study showed that CO_2 enrichment also has notable effects on the yield and fruit weight per plant of oriental melon, with a significant increase under S1 compared to the CK treatment. Moreover, the S1 treatment also resulted in a significant increase in soluble substances compared with CK, highlighting the positive effect of CO_2 enrichment on overall quality. This is thought to be due to the following: first, CO_2 enrichment increases the availability of raw photosynthetic materials, promoting photosynthesis and improving tolerance to environmental stress; second, changes in the leaf structure allow the increased absorption and assimilation of CO_2, promoting the accumulation of organic matter and thus improving the yield and quality [65–67]. In facility production, CO_2 cylinder application equipment can promote plant growth and development, increase the crop growth rate and increase crop yields and benefits. In addition, it is the most intuitive method to improve crop yields and benefits in the greenhouse, with less investment in equipment, and the observed plant yield benefits of increased CO_2 treatment are at least as large as those that can be expected under higher atmospheric CO_2 conditions in the future [68], which is worth considering and applying in the practice of facility agriculture.

5. Conclusions

The results of this study confirm that 1200 μL/L CO_2 enrichment treatment can promote light energy use and CO_2 fixation in oriental melon leaves, enhancing the growth and photosynthetic capacity from day 5 of treatment. We noted a significant increase in the expression of carbon-assimilation-related enzymes from day 15 of treatment, increasing the activity of carbon-assimilation-related enzymes. This, in turn, was found to promote photosynthetic carbon assimilation and organic matter accumulation, significantly increasing the accumulation of nutrients and improving the overall yield and fruit quality. These findings identify the important time node of the CO_2 enrichment of oriental melon, and provide a reference for the high-quality cultivation and off-season cultivation of northern vegetables in solar greenhouses.

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