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The effect of potassium on photosynthetic acclimation in cucumber during CO_2 enrichment

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Abstract

Long-term CO_2 enrichment (1,000 μmol mol^{-1}) leads to photosynthetic acclimation in cucumber. Here, through hydroponic experiments in an open-top climate chamber system, we investigated key photosynthetic parameters of cucumbers using potassium stimulation (120 or 240 mg L^{-1}). Short-term CO_2 enrichment (less than 25 days) significantly increased the net photosynthetic rate in cucumber. However, long-term CO_2 enrichment (43 d) led to photosynthetic acclimation and decrease in stomatal conductance. The increase in potassium alleviated the decrease in photosynthetic rate and stomatal conductance, which reduced photosynthetic acclimation. In addition, ^{13}C isotope tracing showed that under CO_2 enrichment, plants with higher potassium concentrations showed higher sink/source and flow/source ratios of photosynthetic assimilative C ($\delta^{13}C$) abundance. Moreover, the abnormal accumulation of soluble carbohydrates and starch resulted in photosynthetic acclimation in cucumber. Increasing potassium significantly reduced the accumulation of soluble carbohydrates and promoted the transport of soluble carbohydrates to the sink, which alleviated photosynthetic acclimation.

Additional key words: CO_2 enrichment; cucumber; final product negative feedback; photosynthetic acclimation; potassium.

Introduction

Changes in photosynthesis in response to high atmospheric CO_2 concentrations vary with exposure time. Changes due to a short-term exposure (a few minutes to a few days) is usually referred to as a response, whereas those due to long-term exposure (a few weeks to a few months) are usually described as acclimation. Short-term enriched CO_2 exposure increases photosynthetic rate. However, long-term high CO_2 exposure (albeit periodically) gradually reduces the photosynthetic rate of leaves and eventually leads to a lower photosynthetic rate compared to that observed under regular CO_2 concentrations. This phenomenon is defined as photosynthetic acclimation or downregulation. CO_2 enrichment results in an increase in carbohydrates in plants, particularly in leaves (Kimball *et al*. 2002). The massive accumulation of carbohydrates under long-term CO_2 enrichment results in a negative feedback to photosynthesis, which leads to a reduction in the photosynthetic rate and photosynthetic acclimation (Delucia *et al.* 1985). Most of the recent studies on the negative feedback mechanism of photosynthetic acclimation were based on free-air CO_2 enriched (FACE) conditions, namely continuous CO_2 enrichment within a concentration range of 500–700 μmol mol^{-1}. Studies on photosynthetic acclimation and its negative feedback mechanism under CO_2 fertilization conditions (at least 1,000 μmol mol^{-1}, periodically) are limited. In greenhouses, an increase in photosynthetic activity, which occurs at 2–3 h after sunrise, reduces CO_2 concentrations below 100 μmol mol^{-1} that in turn limits the growth of crops due to severe CO_2 deficiency and subsequent photosynthesis suspension (Maggio *et al.* 2002). Therefore, CO_2 fertilization has been widely applied. A study has shown that the optimal CO_2 enrichment concentration is 1,200 μmol mol^{-1}, and the optimal period is 25 d in periodic administration (Lin *et al.* 2011).

Potassium promotes the transport of photosynthetic products to storage organs. In this study, using cucumber as the experimental plant, we investigated whether the accumulation of photosynthetic products in the leaves leads to a negative feedback on photosynthesis in plants that were treated with long-term CO_2 enrichment. Moreover, we evaluated the role of potassium on the transport of accumulated photosynthetic products in cucumber leaves, and whether potassium treatment alleviates photosynthetic acclimation.

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Abbreviations: AC – ambient CO_2 concentration; DAE – days of CO_2 enrichment; EC – elevated CO_2 concentration; FACE – free-air CO_2 enriched; HPLC – high-performance liquid chromatography; g_S – stomatal conductance; P_N – net photosynthetic rate.
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Materials and methods

Locations and materials: The experiment was performed at the farm greenhouse of the Yunnan Agriculture University. The two open-top, plastic film culture chambers inside the greenhouse were 5 m in length, 1.5 m in width, and 1.5 m in height. The experimental cucumber (*Cucumis sativus* L.) cultivar was Zhongnong 20 (from the Institute of Vegetables and Flowers at the Chinese Academy of Agricultural Sciences). The indoor CO_2 concentration was measured using an infrared carbon dioxide detector (*Telaire7001*, *Telaire,* USA). The chamber was supplied with CO_2 (99.9% purity) from a high-pressure steel cylinder after buffering.

Experimental design and protocol: Four groups were used in the experiment. Plants were treated with two different CO_2 concentrations: the control (AC, 380 μmol mol^{-1}) and the CO_2 enrichment (EC, 1,000 μmol mol^{-1}). At each CO_2 concentration, plants were further treated with two different potassium concentrations [120 (K120) and 240 (K240) mg L^{-1}]. For each treatment, nine replicates were performed. A total of 36 plants were studied.

Modified Hoagland medium was used in the experiment. The ingredients were as follows: 261.39 g(K_2SO_4) L^{-1}; 5.22 g(KCl) L^{-1}; 295 g($Ca(NO_3)_2 \cdot 4H_2O$) L^{-1}; 35.51 g($Ca(H_2PO_4)_2 \cdot H_2O$) L^{-1}; 42.11 g(EDTA-Na-Fe) L^{-1}; 160.21 g($MgSO_4 \cdot 7H_2O$) L^{-1}; 0.3905 g($MnSO_4 \cdot H_2O$) L^{-1}; 0.0555 g($ZnSO_4 \cdot 7H_2O$) L^{-1}; 0.0206 g($CuSO_4 \cdot 5H_2O$) L^{-1}; 0.725 g(H_3BO_3) L^{-1}; and 0.0046 g($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$) L^{-1}. A ¼-ion strength Hoagland nutrient solution was prepared by diluting the original medium by 1,000 times.

On 5 March 2016, seeds were germinated in a 25°C incubator with daily morning and night watering. After 48 h, the germinated seeds were planted in culture dishes filled with vermiculite and grown at 25°C. When the plants exhibited three true leaves (27 March), they were transplanted to culture dishes filled with water for one week. On 3 April, ¼-ion strength Hoagland medium was used to replace the water in the culture dishes, and the pH of the medium was adjusted to 6 using 1 N sulfuric acid. When the plants exhibited six true leaves (28 April), ½-ion strength Hoagland medium was used as the culture medium. Young plants of the same size were collected and planted in plastic pots containing 3 L of the culture medium. Each pot contained one plant, with 24-h ventilation and the nutrient medium was replaced every three days. Young plants of the same size were collected and separated into two CO_2 chambers under the following conditions: (*1*) ventilated with regular air (AC, CO_2 concentration of 380 μmol mol^{-1}); (*2*) ventilated with CO_2 enriched air (EC, CO_2 concentration of 1,000 μmol mol^{-1}).

The CO_2 enrichment was made in the morning from 8:00 to 12:00 h. On 4 May 2016, CO_2 enrichment was started; photosynthetic characteristics were measured after 9, 25, and 43 d of CO_2 enrichment (DAE). Samples were also collected at the same time points to evaluate the content of soluble carbohydrates and starch. ^{13}C labeling was performed on 7, 23, and 41 DAE. Approximately 48 h after labeling, namely at 9, 25, and 43 DAE, samples were collected for analysis using the methods demonstrated below.

Photosynthetic rate: The third functional leaf on the top of the plant was collected to evaluate photosynthetic parameters. An open-circuit photosynthetic air analysis system *Li-6400* (*Li-COR*, USA) was used to measure the net photosynthetic rate (P_N) and stomatal conductance (g_s) between 9:00–11:00 h in each chamber.

Photosynthetic product contents: Soluble carbohydrates were measured using high-performance liquid chromatography (HPLC). The chromatographer was from *Waters Corp*., (USA), the detector was an evaporative light detector (*e2695*). The specific chromatographic conditions were as follows; column: Ca column; mobile phase: ultrapure water; flow rate: 0.6 mL min^{-1}; column temperature: 75°C; sample injection volume: 10 μL per sample. The standards for fructose, glucose, and sucrose were purchased from *Amersoc*. The stachyose standard was purchased from *Sigma*. The soluble carbohydrates discussed in this study included fructose, glucose, sucrose, and stachyose.

The measuring method was performed according to Qi *et al*. (2006) and Yu (1985). Briefly, at the indicated time points, three samples from the leaves, stems, roots, and fruits of each plant were collected and stored in a −60°C freezer for further analysis. Approximately 0.3 g of frozen samples was ground and extracted using 50 mL of extraction buffer (99% ethanol). After 10 min of homogenization, the samples were centrifuged at 5,000 × *g* for 15 min, and the supernatants were collected. The extraction, homogenization, and centrifugation processes were repeated thrice, and supernatants from each sample were pooled. The combined supernatants were then dried through reduced pressure evaporation at 40°C. The dried mass was dissolved in 15 mL of deionized water. Five milliliters of chloroform was then added to the solution followed by oscillation, centrifugation, and organic phase removal. This purification process was repeated thrice to remove the phospholipids in the extracts. The pH of the remaining water phase was adjusted to 7 using 0.1 M NaOH, and the solution was dried using a rotary evaporator at 40°C. The dried mass was dissolved using 500 μL of deionized water and then filtered using a 0.45-μm syringe filter prior to analysis.

The extraction and measurement of starch was performed as follows: approximately 2.5 g of the sample was added to a 250-mL round bottom flask and incubated with 80% ethanol overnight. The sample was refluxed in a reflux condenser for 1 h to remove soluble carbohydrates. The remaining biomass was dried at 60°C until no ethanol could be detected and then transferred to another 250-mL round bottom flask. The sample was refluxed and hydrolyzed in the presence of 70 mL of water and 7 mL of hydrochloric acid for 2.5 h in a boiling water bath. The hydrolyzed sample was filtered and then transferred to a 10-mL volumetric flask. One drop of methyl red indicator (5 g L^{-1}) was added to the sample for pH adjustment. The pH value was adjusted to 7 by using 400 g L^{-1} NaOH buffer for coarse adjustment and 10 g L^{-1} NaOH buffer for fine

adjustment. The sample solution was titrated to 10 mL. Two milliliters of the sample solution was filtered using 0.45-μm syringe filter, and 20 μL of the filtered solution was used for analysis.

Carbon isotope tracing: The carbon isotope labeling and analysis were performed according to Yin (2010). Carbon isotope labeling was performed at 7, 23, and 41 DAE, starting at 9:00 a.m. on a sunny day. The labeling isotope was ^{13}C in CO_2. Labeling time was 1 h. While labeling, an infrared carbon dioxide detector *Telaire7001* was used to monitor the concentration of CO_2 to prevent overheating. Approximately 48 h after labeling, samples from the roots, stems, and leaves were collected and pre-treated. After labeling at all above indicated time points was completed, the abundance of isotope $\delta^{13}C$ was measured using the isotope analyzer *Picarro CM-CRDS* (*Picarro*, USA).

Isotope abundance is the percentage of a specific element isotope in the total number of atoms. Usually, the carbon isotope abundance in the soil and plants is expressed as $\delta^{13}C$. The formula is:

$$\delta^{13}C\ (‰) = (R_{sample}/RPDB - 1) \times 1{,}000,$$

where R_{sample} represents the isotope abundance ratio of $^{13}C/^{12}C$ in the sample; and RPDB or $^{13}C/^{12}C$ (PDB) represents the isotope abundance ratio of $^{13}C/^{12}C$ in the standard.

Carbon isotope abundance $\delta^{13}C$ was used to measure carbon isotope content in different cucumber organs.

Biomass content: At 9, 25, and 43 DAE, samples were collected and washed with deionized water, and surface moisture was dried using absorbent paper. The root, stem, leaf, and fruit were weighed and recorded respectively. The samples were incubated at 105°C for 30 min for enzyme deactivation, followed by complete drying at 80°C. The dry mass was weighed and recorded as the total mass of the plant.

Data analysis: One-way and double-way analysis of variance (*ANOVA*) was applied to test difference of dates between different treatments with a 95% confidence level or α = 0.05 and a 99% confidence level or α = 0.01 and mapped by *Origin 8*.

Results

Total biomass: By 43 DAE, the plants treated with AC and K240 exhibited an elevated total biomass compared to the plants treated with AC and K120 (Fig. 1). Furthermore, the total biomass of plants treated with EC and K120 was higher than that of plants treated with ambient CO_2 concentration and K240 (AC-K240). The total biomass of plants treated with EC and K240 was significantly higher than that of any other groups. Our results indicated a synergism between CO_2 concentration and potassium contents on increasing total cucumber biomass.

Net photosynthetic rate and stomatal conductance: From 9 to 25 DAE, P_N significantly increased, which coincided with the observed changes in total biomass. However, by 43 DAE, photosynthetic acclimation occurred (Table 1). The plants treated with EC-K240 showed an increase in P_N compared to plants treated with EC-K120. These findings indicate that both CO_2 concentrations and potassium concentrations acted synergistically in order to regulate the P_N.

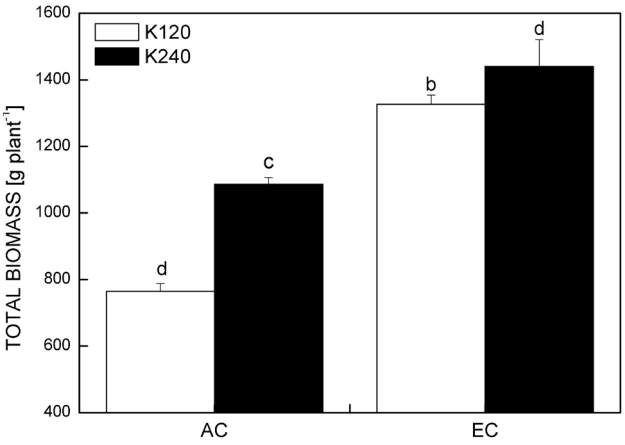

Fig. 1. Effects of elevated CO_2 concentrations and increasing potassium concentrations to total biomass on cucumber plants grown in a greenhouse (95% confidence level or α = 0.05). AC – ambient CO_2 concentration (380 μmol mol^{-1}); EC – elevated CO_2 concentration (1,000 μmol mol^{-1}); K120 – potassium application, 120 mg L^{-1}; K240 – potassium application, 240 mg L^{-1}.

By 9 and 23 DAE, g_s was mainly regulated by potassium concentrations. The g_s under K240 significantly increased compared to that of K120 (Table 2). However, by 43 DAE, g_s under EC significantly decreased, together with a decrease of P_N. Furthermore, elevated potassium concentrations significantly alleviated the decrease in g_s and P_N.

Table 1. Effects of elevated CO_2 and K concentrations on cucumber photosynthetic rates. Results are expressed as the mean ± standard deviation (n = 3). Groups with *different letters* represent significant differences at the same sampling time point between treatments (95% confidence level or α = 0.05). * – significant correlation, 0.05 (two-tail) confidence interval. ** – highly significant correlation, 0.01 (two-tail) confidence interval. P_N – net photosynthetic rate; AC – ambient CO_2 concentration (380 μmol mol^{-1}); EC – elevated CO_2 concentration (1,000 μmol mol^{-1}); K120 – potassium application, 120 mg L^{-1}; K240 – potassium application, 240 mg L^{-1}.

Treatment	P_N [μmol m^{-2} s^{-1}]		
	Day 9	Day 25	Day 43
AC-K120	13.514 ± 0.859^{c}	12.288 ± 1.45^{d}	16.716 ± 0.934^{b}
AC-K240	15.431 ± 0.989^{c}	16.085 ± 0.856^{c}	19.609 ± 0.592^{a}
EC-K120	21.729 ± 1.544^{b}	24.809 ± 0.834^{b}	10.821 ± 1.094^{c}
EC-K240	25.471 ± 0.378^{a}	28.191 ± 0.628^{a}	15.025 ± 1.013^{b}
CO_2	**	**	**
K	**	**	**
CO_2*K	*	**	**

Table 2. Effect of CO_2 and potassium concentrations on the stomatal conductance in cucumber leaves. Results are expressed as the mean ± standard deviation (n = 3). Groups with *different letters* represent significant differences at the same sampling time point between treatments (95% confidence level or α = 0.05). * – significant correlation, 0.05 (two-tail) confidence interval. ** – highly significant correlation, 0.01 (two-tail) confidence interval. g_S – stomatal conductance; AC – ambient CO_2 concentration (380 μmol mol^{-1}); EC – elevated CO_2 concentration (1,000 μmol mol^{-1}); K120 – potassium application, 120 mg L^{-1}; K240 – potassium application, 240 mg L^{-1}.

Treatment	g_s [μmol m^{-2} s^{-1}]		
	Day 9	Day 25	Day 43
AC-K120	0.67 ± 0.02^b	0.56 ± 0.06^b	0.58 ± 0.16^b
AC-K240	0.82 ± 0.03^a	0.65 ± 0.08^a	0.7 ± 0.13^a
EC-K120	0.62 ± 0.1^b	0.52 ± 0.08^b	0.15 ± 0.04^d
EC-K240	0.78 ± 0.01^a	0.57 ± 0.16^a	0.37 ± 0.1^c
CO_2	ns	ns	ns
K	*	*	**
CO_2*K	ns	ns	*

Distribution of photosynthetic assimilative products (^{13}C tracing): By 9, 25, and 43 DAE, the accumulation of photosynthetic assimilative C (δ^{13}C) was highly significantly elevated in the roots in K240 compared to K120 (Fig. 2). The accumulation of δ^{13}C also significantly increased in the stems and leaves, as demonstrated in the same tracing assays. By 9 DAE, K240 increased the accumulation of photosynthetic assimilative C by 5,636.7, 52.7, and 51.7% in the roots, stems, and leaves, respectively, compared to that in plants treated with K120. By 25 DAE, the increase changed to 620.2, 55.0, and 64.7%, respectively. By 43 DAE, the increase changed to 823.6, 113.8, and 49.7%, respectively. A significant difference in accumulation between K120 and K240 was observed in the stems and leaves and a highly significant difference was observed in the roots.

The effect of CO_2 enrichment and high potassium concentrations on the sink (root)/source (leaf) ratio and the flow (stem)/source (leaf) ratio of δ^{13}C abundance is shown in Table 3. After EC, the sink/source ratio and flow/source ratio of δ^{13}C at K240 was higher than that of δ^{13}C at K120. In addition, the sink/source ratio and flow/source ratio of δ^{13}C in plants treated with K240 increased at the three time points tested, particularly by 25 and 43 DAE.

Accumulation of photosynthetic assimilative products (starch and soluble carbohydrates): The plants treated with EC-K240 exhibited a higher root/leaf ratio of soluble carbohydrate content compared to the other groups (Table 4). The plants treated with EC-K240 exhibited a lower root/leaf starch content ratio compared to the other groups (Table 5).

Correlation analysis between P_N and the content of soluble carbohydrates and starch: The four groups exhibited a negative correlation between P_N and starch content. The plants treated with EC and K240 exhibited a highly significant, negative correlation between P_N and starch content (Table 6).

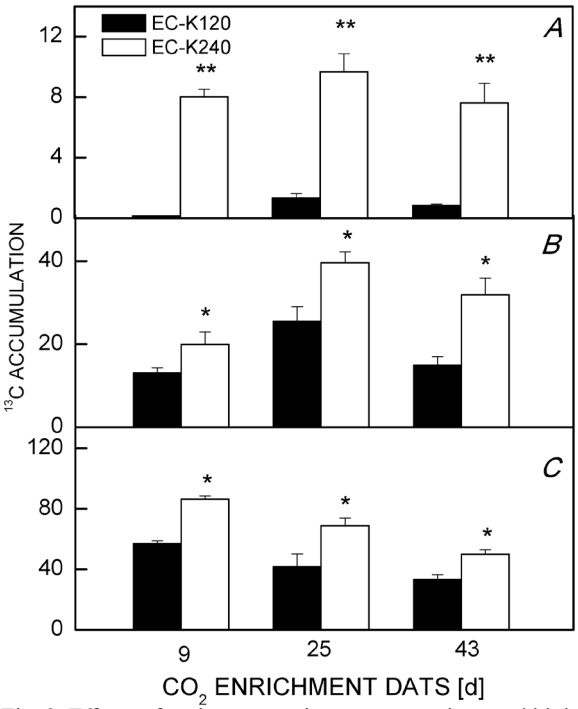

Fig. 2. Effects of various potassium concentrations and high CO_2 concentrations on C (δ^{13}C) accumulation in the roots, stems, and leaves of cucumber plants. *Significant correlation, 0.05 (two-tail) confidence interval. **Highly significant correlation, 0.01 (two-tail) confidence interval. AC – ambient CO_2 concentration (380 μmol mol^{-1}); EC – elevated CO_2 concentration (1,000 μmol mol^{-1}); K120 – potassium application, 120 mg L^{-1}; K240 – potassium application, 240 mg L^{-1}.

Discussion

CO_2 is the main component of dry mass in plants and it has been widely reported that CO_2 enrichment increases plant biomass. For example, Agüera *et al*. (2006) showed that CO_2 enrichment increases the total biomass of cucumber plants. Using elevated CO_2 and potassium conditions in cotton, Reddy *et al*. (2005) showed that increasing potassium contents in the soil promotes dry mass accumulation in plants, which acts synergistically with higher CO_2 concentrations. In the present study, we showed that CO_2 enrichment increased the biomass of cucumber, and the increase in potassium contents further elevated the total biomass of cucumber. Thus, we conclude that CO_2 acted synergistically with potassium and increased cucumber biomass.

Short-term (several days) CO_2 enrichment increases the photosynthetic capability of plants, which was reflected in the present study by an increase in P_N and g_s. However, long-term (several weeks) CO_2 enrichment induces different responses in plants (Long *et al.* 2004). Studies on photosynthetic acclimation under FACE conditions have been developed, whereas investigations on CO_2 fertilization conditions (high concentration, periodic administration)

Table 3. The effect of CO_2 and potassium on the ratio of C abundance ($\delta^{13}C$) sink (root)/source (leaf), flow (stem)/source (leaf) ratio to cucumber. AC – ambient CO_2 concentration (380 μmol mol^{-1}); EC – elevated CO_2 concentration (1,000 μmol mol^{-1}); K120 – potassium application, 120 mg L^{-1}; K240 – potassium application, 240 mg L^{-1}.

Treatment	Sink (Root)/ Source (Leaf)			Flow (Stem)/ Source (Leaf)		
	9	25	43	9	25	43
EC-K120	0.0025	0.032	0.025	0.18	0.46	0.45
EC-K240	0.093	0.14	0.15	0.23	0.57	0.64

Table 4. Effects of CO_2 and potassium supply on the ratio of soluble sugar for cucumber roots/leaves. AC – ambient CO_2 concentration (380 μmol mol^{-1}); EC – elevated CO_2 concentration (1,000 μmol mol^{-1}); K120 – potassium application, 120 mg L^{-1}; K240 – potassium application, 240 mg L^{-1}.

Time [d]	Root/leaf soluble sugar content ratio			
	AC-K120	AC-K240	EC-K120	EC-K240
9	0.35	0.35	0.54	0.51
25	0.42	0.46	0.55	0.71
43	0.56	0.58	0.65	0.72

Table 5. Effects of CO_2 and potassium supply on the ratio of starch for cucumber roots/leaves. AC – ambient CO_2 concentration (380 μmol mol^{-1}); EC – elevated CO_2 concentration (1,000 μmol mol^{-1}); K120 – potassium application, 120 mg L^{-1}; K240 – potassium application, 240 mg L^{-1}.

Time [d]	Root/leaf starch content ratio			
	AC-K120	AC-K240	EC-K120	EC-K240
9	0.90	0.92	0.84	0.83
25	1.14	0.78	0.69	0.66
43	0.81	0.8	0.72	0.56

Table 6. Correlation between net photosynthetic rate, glucose, and starch content in cucumber leaves. AC – ambient CO_2 concentration (380 μmol mol^{-1}); EC – elevated CO_2 concentration (1,000 μmol mol^{-1}); K120 – potassium application, 120 mg L^{-1}; K240 – potassium application, 240 mg L^{-1}. * – significant correlation, 95% confidence level or α = 0.05 (two-tail). ** – highly significant correlation, 99% confidence level or α = 0.01 (two-tail).

Treatment	P_N -soluble sugar	P_N -starch
AC-K120	0.489	−0.753
AC-K240	0.701	−0.555
EC-K120	0.758	−0.612
EC-K240	0.930**	−0.983**

are limited. The present study showed that on 9 and 25 DAE, the P_N of plants periodically treated with CO_2 at 1,000 μmol mol^{-1} significantly increased compared to that of control plants under regular environmental conditions. However, on 43 DAE, the P_N of plants treated with 1,000 μmol(CO_2) mol^{-1} of was significantly lower than that of the control plants under regular environmental conditions. Our previous study showed that on 27 DAE, the P_N of plants treated with CO_2 at 1,000 μmol mol^{-1} was the same as that of the control plants under regular environmental conditions (data not shown). Similar to the data in FACE conditions, our results showed that periodic administration of EC (1,000 μmol mol^{-1}) leads to photosynthetic acclimation. We also showed that an increase in potassium concentrations improves the photosynthetic rate. In addition, in each of the two CO_2 concentrations, the elevated potassium content did not result in a significant increase of the photosynthetic rate on 9 DAE, although this was observed on 25 and 43 DAE. Thus, we concluded that high potassium content induced an increase in the photosynthetic rate of plants subjected to long-term high CO_2 exposure, as well as alleviated photosynthetic acclimation.

Studies on FACE conditions have demonstrated that photosynthetic acclimation in wheat and rice is not due to stomatal limitation, namely, changes in stomatal conductance (Liao *et al.* 2002, 2003). However, other studies have shown that changes in CO_2 concentrations affect stomatal conductance (Chen *et al.* 2011). Our data show that short-term CO_2 enrichment (on days 9 and 25) did not affect g_s, whereas an increase in potassium concentration enhanced g_s under two CO_2 concentrations. However, long-term CO_2 enrichment (on 43 DAE) significantly reduced g_s, whereas an increase in potassium content alleviated the decrease in g_s. Thus, photosynthetic acclimation may be correlated with stomatal limitation, and potassium contents play a significant role in this particular process. This observation has also been validated through other CO_2 fertilization experiments (Yuan *et al.* 2009).

Several studies have shown that increasing CO_2 concentration improves the transport of photosynthetic products to the roots. Other studies have also demonstrated that CO_2 concentration does not affect the allocation of photosynthetic products (Xu *et al*. 2007). In this study, we performed ^{13}C isotope tracing experiments at different time points after CO_2 enrichment and investigated the allocation of photosynthetic products in cucumber plants exposed to different potassium concentrations. Our results demonstrated that elevated potassium contents significantly promoted the accumulation of photosynthetic products in different plant organs. At the three indicated time points (9, 25, and 43 DAE), high potassium treatment significantly increased the abundance of photosynthetic assimilative C ($\delta^{13}C$) in the roots, stems, and leaves, in which the increase in the abundance in the roots was significant. This suggested that the elevation in potassium increases transport of photosynthetic products from the leaves (source) to the roots (sink). The elevated potassium concentrations ensured the transport of photosynthetic products from the source to the sink, particularly on 43 DAE, when the photosynthetic acclimation occurred. This indicates that increasing potassium under CO_2 enrichment conditions promotes the transport of photosynthetic products, which allocates the photosynthetic products to different plant organs and consequently alleviates

photosynthetic acclimation. This is also validated by the observed photosynthetic assimilative C ($\delta^{13}C$) sink (roots)/source (leaves) and flow (stems)/source (leaves) ratios. At the three indicated time points, both the sink/source and flow/source ratios increased when potassium increased under the CO_2 enrichment conditions. This demonstrated that elevating potassium contents under CO_2 enrichment conditions promotes the transport of photosynthetic products from the source (leaf) to the flow (stem) and the sink (root) of cucumber plants.

In plants, massive starch accumulation in leaves leads to a pressure gradient between leaves and roots, which in turn promote soluble carbohydrate transport (Suter *et al.* 2002, Xu *et al.* 2007). The root (sink)/leaf (source) soluble carbohydrates content ratio of plants treated with AC and K120 was lower than those treated under the other conditions. Particularly, on 43 DAE, the root (sink)/leaf (source) soluble carbohydrates content ratio of plants treated with EC and K240 was the highest. This suggests that high potassium treatment promotes the transport of photosynthetic products from the leaves (source) to the roots (sink). The root/leaf starch content ratio of plants treated with regular CO_2 and potassium contents was higher than those in the other conditions, which is opposite that observed in the root/leaf soluble carbohydrates content ratio described earlier. Thus, CO_2 enrichment and potassium enhancement promoted photosynthesis, starch accumulation, and the allocation of soluble carbohydrates.

In this study, the net photosynthetic rates of plants treated with all four conditions were negatively correlated with the corresponding starch contents. In particular, the net photosynthetic rate of plants treated with high potassium and high CO_2 was highly significantly and negatively correlated with the starch content. This suggests that long-term CO_2 enrichment leads to massive starch accumulation in the leaves, which in turn impairs photosynthesis and reduces net photosynthesis. However, potassium elevation increases starch accumulation, enhances the osmotic pressure between source and sink, and promotes the transport of photosynthetic products from the source to the sink. The root/leaf soluble carbohydrates content ratio of plants treated with regular CO_2 and regular potassium concentrations was lower than that for plants subjected to the other conditions. In addition, on days 25 and 43 of CO_2 enrichment, the root/leaf soluble carbohydrate content ratio of plants treated with high CO_2 and high potassium was the highest. Our data suggest that high potassium contents under CO_2 enrichment conditions promote the transport of photosynthetic products from the leaves (source) to the roots (sink).

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