Original Research

The Effect of Elevated CO_2 Concentrations on Leaf Carbohydrate, Chlorophyll Contents and Photosynthesis in Radish

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Abstract

The objective of this study was to evaluate the effect of different CO_2 concentrations on carbohydrate, chlorophyll contents and net photosynthetic productivity in radish (*Raphanus sativus* L., cv. *Žara*) leaves. Plants were exposed to 350, 700, 1,500 and 3,000 ppm atmospheric CO_2 concentrations over ten days. Day/ night temperature was 24°C/17°C and photoperiod was 16 h. Carbohydrate (fructose, glucose, sucrose and maltose) content analysis was performed using chromatographic methods. The content of chlorophyll was evaluated spectrophotometrically. The results showed that elevated CO_2 increases total carbohydrate content and changes in hexoses/sucrose ratio. A significant increase in chlorophyll content was only in 1,500 ppm treatment. Differences in photosynthetic productivity rate were within error margins. There was no effect on carbohydrate and chlorophyll contents in radish leaves seven days after returning plants to ambient CO_2, though higher photosynthetic productivity rate was in radish, previously grown under 700 ppm CO_2. In summary, leaf carbohydrate contents affect the intensity of photosynthetic pigment synthesis.

Keywords: radish, carbon dioxide, carbohydrates, net photosynthetic productivity

Introduction

The mechanism of plant acclimation to a changeable environmental condition is a major scientific problem. Due to anthropogenic activities CO_2 concentration in the atmosphere is steadily increasing, which affects on plant physiology, morphogenesis, and photosynthesis. There is currently no general model to predict plant acclimation to a varied environment, because the response to elevated carbon dioxide concentration is dependent on species, on plant development stage and can be modified by a number of factors, including light, nutrient and water availability [1]. Biochemical and molecular responses are not well documented yet due to a lack of understanding about the regulatory mechanisms, metabolic signaling and phytochemical changes in plants under elevated CO_2 conditions.

Short-term exposure of elevated CO_2 for plants generally leads to increased rates of leaf-level photosynthesis due to enhanced activity of ribulose-1.5-bisphosphate carboxylase/oxygenase (Rubisco) [2]. The response to elevated CO_2 results in an increase in leaf area, biomass accumulation or individual plant size [3-6]. The rate of CO_2 assimilation by photosynthesis at twice ambient CO_2 concentration may increase by 50% or more in the short term [2]. Long-term CO_2 exposure may lead to adaptation mechanisms in plants [1] and photosynthetic responses are less consistent. In response to elevated CO_2, photo-

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synthetic capacity has been found to decrease, increase, or show no effect, depending on plant tissue types, species, or experimental conditions [4].

As the result of elevated CO_2, carbohydrates are accumulated in plant tissues, as their use intensity is lower than the production under these conditions [7, 8]. Previous studies have reported that during growth under twice-ambient CO_2, leaf soluble carbohydrate content increased on average by 52% and starch content by 160% [2]. An increase in leaf carbohydrates has long been associated with an inhibition of photosynthesis due to a decrease in content and activity of Rubisco protein, which is responsible for CO_2 fixation [7, 9, 10]. Growth under elevated carbon dioxide generates increased leaf levels of sucrose and intense hydrolytic decomposition into fructose and glucose through acid invertase [7]. The resulting hexoses are phosphorylated by hexokinase, which acts as a sugar sensor: it initiates a signal cascade that results in the repression of a number of photosynthetic genes [7, 11]. As a result, a decrease in leaf chlorophyll, nitrogen content, and Rubisco activity is observed [12]. Glucose and maltose may be produced as potential sources of signals primarily by sucrose cycling and secondarily by starch hydrolysis. However, the main role in photosynthesis inhibition processes is attributed to sucrose cycling [2].

Previous studies dealing with high CO_2 exposure on various plant species correlate the decrease in photosynthetic capacity with total insoluble carbohydrates and starch content. However, currently there is no solid basis to predict plant acclimation to elevated CO_2. Therefore, the object of this study was to evaluate the exposure of different CO_2 concentrations on carbohydrate quantity and photosynthetic parameters in radish (*Raphanus sativus* L., cv. *Žara*) leaves.

Materials and Methods

Experiments were performed during 2005 in the phytotron complex of the Lithuanian Institute of Horticulture (Babtai, Lithuania). Radish (*Raphanus sativus* L., cv. *Žara*) plants were sowed on the 4th of August, in peat substrate, 25-30 seeds per 5 L pot. Until germination and one week after, plants were grown in a greenhouse. Temperature in the greenhouse was $25\pm3°C$ and the light source was natural solar radiation. Photosintetically active radiation was about 18,000 MJ m². On the 15th of August pots were transferred to phytotron chambers with 16 h photoperiod and 24/17°C day/night temperature. Light was provided by SON-T-Agro (Philips, USA) lamps. For ten days carbon dioxide concentration in phytotron chambers was maintained at 350, 700, 1,500 and 3,000 ppm. After ten days plants were transferred back to the greenhouse. Ambient CO_2 concentration in the greenhouse was about 370 ppm.

After 10 days of CO_2 exposure and after one week after returning the plants back to the greenhouse, leaf area, plant height, and dry and green matter were measured. Biometric measurements were performed in five replications. Chlorophyll and carbohydrate content in the leaves was also estimated. Total chlorophyll content in green matter was determined in 100% acetone extracts using the spectrophotometrical Wettstein method [13], with a Genesys 6 spectrophotometer (ThermoSpectronic, USA). Three biological samples were measured and the standard deviation was presented in Fig.2 as the error bars.

Carbohydrate samples were prepared by grinding about 1g of leaf fresh matter (FM) and extracted with 4 mL hot bidistilled water. After 24 h extract was filtered through cellulose and membrane (pore diameter 0.2 μm) filters. Chromatographic analysis was carried out using a Shimadzu 10A HPLC system with refraction index detector (Shimadzu, Japan) and Adsorbosil NH_2- column (150 mm x 4.6 mm; Alltech, USA), with mobile phase of 75% aqueous acetonitrile and flow rate of 1 mL/min. Data error bars presented in Fig.1 are the standard deviations of five analytical measurements.

Net photosynthetic productivity was estimated from the plant dry mass and leaf area according to Květ [14].
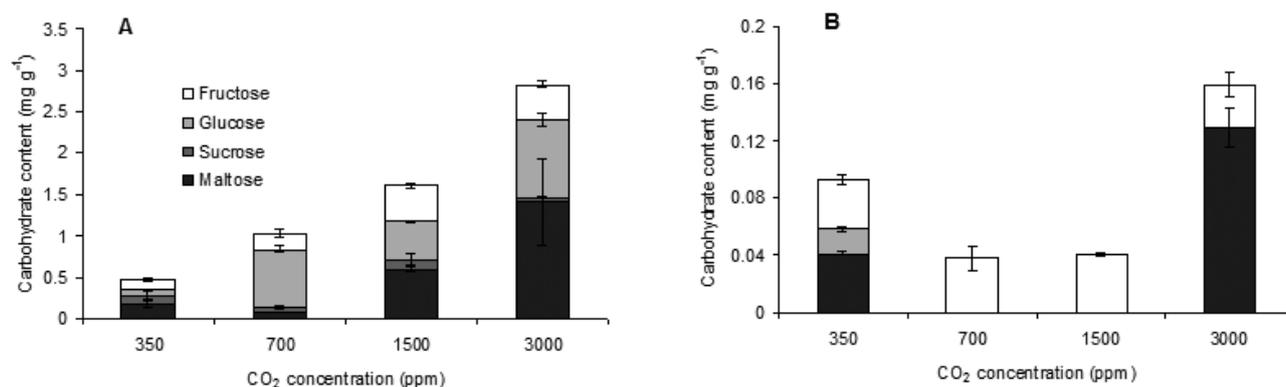

Fig. 1. Carbohydrates content in leaves of radish grown under different CO_2 concentrations.
A – measurements were performed immediately after 10 days of CO_2 exposure; B – measurements were performend 7 days after plants were transfered to ambient CO_2 in the greenhouse.

Results

Carbohydrate measurements after ten days CO_2 exposure showed that total content of carbohydrates is greater at higher carbon dioxide concentrations. Glucose and fructose contents were about three times higher at 700-3,000 ppm CO_2 concentration (Fig.1 A). Maltose content rose remarkably at 1,500 and 3,000 ppm (Fig.1 A). A significant increase in total chlorophyll content was observed when plants were exposed to 1,500 ppm CO_2 (Fig.2 A). Differences in net photosynthetic productivity (Fig. 3 A) are nonsignificant, although there is a trend for the productivity rate to be greater at higher CO_2 concentrations than control.

One week after the plants returned to the greenhouse, total content of carbohydrates (Fig.1 B) were about 30 times smaller then immediately after high CO_2 exposure. There was no sucrose in all treatments, including control. Glucose contents were negligible. Fructose quantity was similar in all treatments and similar to the content immediately after high CO_2 concentration exposure. Maltose was detected in the control treatment and in the 3,000 ppm CO_2 concentration treatment, where its contents were 3 times higher. There were small differences in chlorophyll content among treatments (Fig. 2 B). Photosynthetic productivity was statistically higher in radish, which previously experienced 700 ppm carbon dioxide exposure (Fig. 3 B). Differences among other treatments were within error bars.

Discussion

Carbohydrates are the energy source for most plant physiological processes such as respiration and cell growth. Sugars have important hormone–like functions as primary messengers due to their essential role in plant growth, development and metabolic links with primary physiological processes [11]. The hypothesis that increased carbohydrate contents in plant tissue affects repression of genes, encoding expression of Rubisco and other photosynthetic proteins under elevated CO_2 conditions has been proved by number of previous studies [2, 7-9].

Our results showed that leaf carbohydrate content rise at elevated atmospheric CO_2 concentrations. The most considerable differences were observed in maltose content, the product of starch hydrolytic degradation. However, the major role in photosynthesis regulation is attributed to glucose, fructose and sucrose [1]. According to a previous hypothesis, sucrose cycling is a key path for carbohydrate signaling. Enzyme hexokinase, which participates in the phosphorylation of sucrose's metabolic products (fructose and glucose), also acts as a "sugar sensor" [7]. The activity of this enzyme affects the expression of photosynthetic genes. An important parameter is the hexoses/sucrose ratio, because it reflects changes in carbohydrate metabolism and hexokinase activity. In our study, the hexoses/sucrose ratio at 1500 ppm CO_2 concentration was about 10 and at 7,000
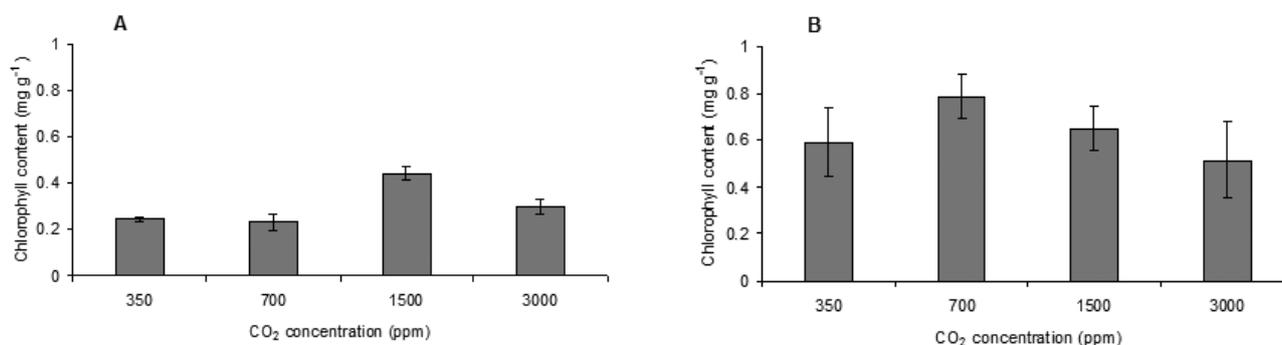

Fig. 2 Total chlorophyll content in radish leaves, grown under different CO_2 concentrations.
A – measurements were performed immediately after 10 days of CO_2 exposure; B – measurements were performed 7 days after plants were transfered to ambient CO_2 in the greenhouse.
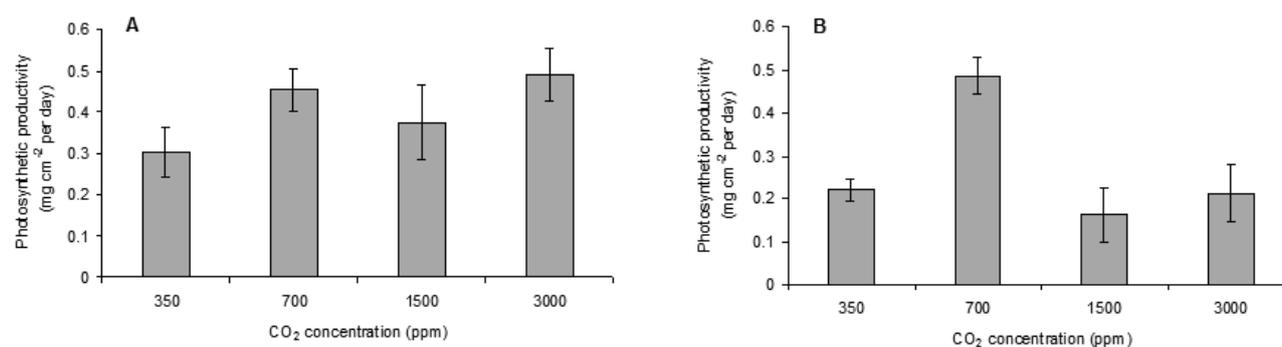

Fig. 3 Photosynthetic productivity in radish leaves, grown under different CO_2 concentrations.
A – measurements were performed immediately after 10 days of CO_2 exposure; B – measurements were performend 7 days after plants were transfered to ambient CO_2 in the glasshouse.

and 3,000 ppm concentrations it was about 20. Comparing these results with chlorophyll content, the previous assumption is proved. Chlorophyll content in radish, which experienced an exposure of 1500 ppm CO_2 was remarkably higher than in other treatments.

Photosynthetic productivity rate in our experiments was higher in radish grown under 700-3000 ppm of CO_2. Plant growth and net primary production depend on the balance between carbon gain through photosynthesis and carbon loss through respiration. Elevated CO_2 often increases leaf area, but the extent of this stimulation depends on species and other environmental variables [5]. A review of the literature [6] suggested that cell division and cell expansion may be affected, mainly due to increased substrate (sucrose) availability and perhaps also due to differential expression of specific genes [15].

Analysis one week after returning the plants to the greenhouse showed contradictory results. Total carbohydrate content was lower in all treatments compared to the measurement immediately after the 10 days of elevated CO_2. Total chlorophyll content, on the other hand, was about 50% higher than immediately after CO_2 exposure. Such changes and the reduced photosynthetic productivity rate could be associated to plant aging (the developmental stage) when the samples were taken. It has been reported that photosynthetic acclimation does not take place in young plants [2] and glucose and fructose content increase with leaves age when starch content decreases [11]. In our study fructose concentration in plants grown under elevated CO_2 decreased about 70-90% 10 days after the elevated CO_2 treatments ended and glucose was found only in the control treatment. A glucose concentration decrease to a critical level may have significant effect on cell and leaf growth, because it is the main plant metabolite, the substrate for respiration and structural unit of starch and cellulose synthesis. As reported previously, the increased starch accumulation under elevated CO_2 conditions affects sucrose metabolism and causes the decrease in glucose content [16].

The change in atmospheric CO_2 concentration affects the balance of carbohydrate metabolism. However, more detailed analysis, including Rubisco content, enzyme activity and gene determination is necessary to relate carbohydrate accumulation with changes in the photosynthetic apparatus.

Conclusions

Exposure of elevated CO_2 led to an increased carbohydrate production and metabolism and to increased rates of leaf–level photosynthesis. Quantitative hexoses/sucrose ratio affects intensity of photosynthetic pigments synthesis depending on atmospheric CO_2 concentrations.

The decrease in carbohydrate content, changes in chlorophyll content and photosynthetic productivity seven days after returning plants to ambient carbon dioxide are not related to previous CO_2 exposure.

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