Physiological regulation of soybean (Glycine max L. Merr.) growth in response to drought under elevated CO₂

Dongxiao Li 1, 2, Huiying Liu 3, Yunzhou Qiao 1, Youning Wang 4, Baodi Dong 1, Zhaoming Cai 4, Changhai Shi 1, Yueyan Liu 1, 2, Xia Li 4* and Mengyu Liu 4*

1 Key Laboratory of Agricultural Water Resources & Hebei Key Laboratory of Agricultural Water-Saving, Centre for Agricultural Resources Research, Institute of Genetic and Developmental Biology, Chinese Academy of Sciences, Shijiazhuang, 050021, China. 2 University of Chinese Academy of Sciences, Beijing 1000439, China. 3 Shijiazhuang Center for Agricultural Product Quality Inspection, Shijiazhuang city, 050021, P.R. China. 4 Present address: Center for Agricultural Resources Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, No. 286, Huaizhong Road, Shijiazhuang, 050021, P. R. China. *e-mail: mengyuilitj@163.com, yunzhouqiao@hotmail.com, youningwang@163.com, dongbaodi@126.com, caizhaoming-2000@163.com, chhshi@sjziam.ac.cn, liuyueyan3544@126.com, xli@genetics.ac.cn

Received 8 January 2013, accepted 20 April 2013.

Abstract

To understand the physiological mechanism by which soybean (cultivar: Glycine max L. Merr. cv.JH13) regulates its growth in response to drought under elevated CO₂ concentration, a pot experiment with two levels of CO₂ (350 vs. 700 μmol·mol⁻¹) and two levels of water (well irrigated vs. drought) was conducted. Plant relative growth rate (RGR), water potential (WP), photosynthetic pigment (chlorophyll a/b, chlorophyll, and carotenoid contents), net photosynthetic rate (Pn), total soluble protein and total soluble sugar content, antioxidant enzyme activities (total superoxide dismutase, Cu-Zn superoxide dismutase, and peroxidase), and malondialdehyde (MDA) content in leaves were measured across different reproductive growth stages. The results revealed that elevated CO₂ concentration increased RGR by 8.7 and 55.6% under well-irrigated and drought conditions, respectively. Elevated CO₂ concentration reduced the decrease in water potential and Pn by 8.3 and 14.9% under drought condition, respectively, but chlorophyll content, chlorophyll a/b, and carotenoid content were not influenced obviously in enrich CO₂ irrespective of water status. At elevated CO₂, more soluble sugar and soluble protein accumulated under drought condition than under normal water condition, suggesting osmotic regulation. Antioxidant enzyme activities were slightly stimulated under elevated CO₂ concentration alone, but membrane-lipid peroxidation was not alleviated significantly in the combination treatment of elevated CO₂ and drought. It was possible that enzyme responses to enhanced CO₂ level were found to vary with the plant species and different phenophases. These results could be used to study of crops response to future climate change, and provide some reference values for agriculture irrigation in the North China Plain.

Key words: Elevated CO₂ concentration, drought, net photosynthetic rate, water potential, relative growth rate, antioxidant enzyme activity, osmotic adjustment.

Introduction

CO₂ elevation, one of the main inducers of climate change, is often accompanied by changes in precipitation. According to the Intergovernmental Panel on Climate Change (IPCC) 14, CO₂ concentration has increased at an unprecedented rate since the industrial revolution and will surpass 700 μmol·mol⁻¹ by the end of the 21st century. One of the outcomes of global climate change is drought, which has raised widespread concern regarding food supply for the world’s increasing population. Periods of drought also tend to be longer, causing an increasingly severe problem in cereal production 6. Great annual yield losses in soybean, one of the most important economic crops in the North China Plain, have been reported by Sincik et al. 35. Exposure of plants to adverse conditions often inhibits the normal growth of plants and causes oxidative stress, such as the production of active oxygen species 21. Accordingly, plants activate defense mechanisms that are regulated by multiple physiological processes to avoid harmful effects 40.

Accumulating evidence strongly indicates that elevated CO₂ can accelerate plant growth and development. Plants respond to CO₂ on the ecosystem, community, population, physiological, and molecular scales 36, 37. However, an early report has shown that elevated CO₂ significantly decreases antioxidative enzyme activities, chlorophyll, carotenoids, and total soluble protein contents in two soybean genotypes grown in open-top chambers 39. Recent studies have reported that elevated CO₂ mitigates the degree of change in all physiological factors under drought stress and improves the drought tolerance of tall fescue 41. In addition, the effects of enhanced CO₂ on plant physiology have been confirmed to be influenced by the developmental stage of the plant and by water deficit 16, 22. Longer exposure to high CO₂ often results in photosynthetic acclimation, which is related to sink-source status, leaf rubisco activity, and protein content 3, 16. C₃ and C₄ crops have diferent responses to enriched CO₂ concentration, but the beneficial effects of elevated CO₂ on both
ameliorate the adverse affects of drought stress. Although enormous researches have shown that elevated CO₂ concentration generally promotes crop growth and increases yield, the effect of elevated CO₂ on plant response to drought stress is still under debate. To date, physiological effects associated with elevated CO₂ and drought on soybean plants grown in the Huang-Huai-Hai Plain of China remain unclear.

In this study, we used a conventional soybean cultivar to further investigate the effectiveness of elevated CO₂ in mitigating the negative effects of drought and to identify the regulatory mechanism of physiological components at the leaf level. We hypothesize that elevated CO₂ could improve growth and cause a relaxation of the antioxidant defense system of plant leaves under drought condition. The results of this study may serve as a theoretical basis for the physiological mechanism by which elevated CO₂ facilitates soybean growth regulation under drought and for the early selection of soybean varieties with high water use efficiency.

Materials and Methods

Growth conditions: Experiments were conducted in six enclosed top chambers (ETCs; 3 mm thick glass) at Luancheng Agro-Eco Experimental Station, Chinese Academy of Sciences (37.53 N, 114.41 E; altitude, 50.1 m). Air temperatures inside the chambers were controlled by a temperature-controlling system consisting of a heater, cooler, and temperature transmitter, and the inside temperature was maintained the same as that outside the chamber. Two CO₂ concentrations (Ambient: 380±32.2 µmol·mol⁻¹ and Elevated: 740.6±45.4 µmol·mol⁻¹) inside the ETCs was continuously monitored and controlled electronically (Fig. 1). Pure CO₂ gas was diluted to 3000 µmol·mol⁻¹ with air before being injected into the CO₂-enriched ETCs.

Planting material and watering levels: Soybean (Glycine max, cv. JiHuang13) seeds were sown on June 20 in combined medium of vermiculite and perlite (volume ratio = 3:1) in 6.18- litre (20 cm × 20 cm) pots. The growing medium saturated with a nutrient solution. About 3 days after, seedlings emerged and were thinned out to the required population density (3 plants per pot; 16,000 plants per acre).

Soil volumetric water contents were monitored using a Wet sensor (Wet 2; MIDWEST-G; Britain) and maintained at drought (30–40% RWC) or normal (60–70% RWC) level through irrigation during the growing season. Sixteen pots were randomly assigned to normal water and drought treatments, and thirty-two pots were placed inside each ETC. Four treatments was set in growth chambers: (1) elevated CO₂ and normal water status (EN); (2) elevated CO₂ and drought (ED); (3) ambient CO₂ and normal water status (AN); and (4) ambient CO₂ and drought (AD).

Leaf water potential and gas exchange: Fully unfolded, central leaflets (functional leaves, often the third trifoliate leaf) were selected and used to measure Pn and vapor pressure deficit using a Li-6400 portable photosynthesis system (LI-COR, Lincoln, NE, USA) at 25±0.3°C, with a radiation intensity of 1500 µmol·m⁻²·s⁻¹ between 9:30 am and 11:00 am. A WAP4 Dewpoint Potential Meter (Decagon Devices, Inc., Pullman, WADC, USA) was used to determine leaf water potential (WP) during midday conditions. The process was repeated five times for each sample.

Measuring photosynthetic pigments: The third trifoliolate leaves were sampled from three randomly selected soybean plants in each plot. Triplicate samples of leaf tissues (0.1 g) were excised and then soaked in 10 ml of 95% ethanol for 24 h in the dark to extract chlorophyll. The supernatant obtained was used to assay the chlorophyll and carotenoid contents through colorimetric determination with a UV-2450 spectrophotometer (Shimadzu) at colorimetric wavelengths of 665, 649 and 470 nm. The photosynthetic pigments contents were calculated as previously described by Lichtenthaler and Wellburn.

Assay of superoxide dismutase (SOD) and peroxidase (POD) activities in leaves: Leaves from each treatment were collected in liquid nitrogen and stored at -80°C prior to extraction. Leaf tissues (0.5 g) were homogenized in an ice bath in 1 ml of 0.1 M sodium phosphate buffer (50 mM, pH 7.8) containing 1% polyvinyl pyrrolidone and then centrifuged at 10,000 g for 15 min at 4°C. The supernatant was used for the estimation of total SOD, Cu-Zn SOD, and POD activities.

Total SOD and CuZn-SOD activities were measured based on the nitroblue tetrazolium reduction method. The reaction mixture was determined by a UV-2450 spectrophotometer (Shimadzu) at a colorimetric wavelength of 550 nm. POD activity was detected ac-cording to the method described by Sakharov and Ardila with slight modifications: 2.91 mL phosphate buffer (10 mM, pH 7), 0.05 mL guaiacol (20 mM), 40 mM H₂O₂, and 0.02 ml enzyme solution. POD activity was measured at a colorimetric wavelength of 420 nm. The activities of total SOD, Cu-Zn SOD, and POD were expressed as enzyme units per milligram protein (U mg⁻¹ protein).

Figure 1. Two CO₂ concentrations during experiment stage: AC, ambient CO₂ concentration (380.1 ± 32.2 µmol mol⁻¹); EC, elevated CO₂ concentration (740.6 ± 45.4 µmol mol⁻¹).

Figure 1. Two CO₂ concentrations during experiment stage: AC, ambient CO₂ concentration (380.1 ± 32.2 µmol mol⁻¹); EC, elevated CO₂ concentration (740.6 ± 45.4 µmol mol⁻¹).
the methods described by Cakmak.

Samples of leaves (0.5 g) were extracted with 10 ml of 6 M HCl and 15 ml of boiling H2O for 20 min, and then diluted with distilled water to 100 ml. Approximately 4 ml of anthrone reagent was then added to 1 ml of the prepared solution in a clean dried test tube. The solution was cooled rapidly and then subjected to colorimetric determination with a U-2001 spectrophotometer (Shimadzu) at a colorimetric wavelength of 620 nm. The total soluble sugar, soluble protein, and MDA contents were calculated and expressed as mg/g fresh weight.

Crop growth analysis methods: Relative growth rate (RGR) was also estimated following the methods described by Shipley. 

\[
RGR = \frac{NAR \times SLA \times LMR}{LW}\]

where NAR is the net assimilation rate (g cm\(^{-2}\) day\(^{-1}\)), a measure of the increase in a plant dry weight per leaf area per day from the flowering to seed-filling stage; LMR is the leaf mass ratio (g g\(^{-1}\)); a measure of biomass allocated to leaves; and SLA is the specific leaf area (cm\(^{2}\) g\(^{-1}\)), which was calculated from the ratio of leaf area (cm\(^{2}\)) to leaf dry weight (g). Leaf area was measured using a leaf area meter (LI-3100; LI-COR, USA). The leaves were oven dried and weighed.

Data analysis: The experimental design was a completely randomized split plot design. All data were subjected to ANOVA using SPSS version 17.0. Each pot from an ETC was considered as an individual replicate. Tukey’s multiple comparison test at \(\alpha = 0.05\) probability level was used to determine the differences among mean values.

Results and Discussion

RGR of soybean under elevated CO\(_2\) and two water conditions:

On average across all the reproductive growth stages, elevated CO\(_2\) concentration increased RGR by 8.7% \((P<0.05)\), significantly increased NAR by 39%, but decreased LMR by 21.9% (Table 1). Drought decreased NAR, SLA, LMR, and RGR under the two CO\(_2\) concentrations. However, RGR and NAR in the combination treatment of elevated CO\(_2\) and drought were 55.6 and 33.4% \((P<0.05)\) higher than those in the drought treatment alone, respectively. This finding may be related to the increased substrate supply and/or increased water stress alone. This is inline with the results of more biomass produced under elevated CO\(_2\) and drought conditions.

Table 1. Analysis of variance of net assimilation rate (NAR, g cm\(^{-2}\) day\(^{-1}\)), specific leaf area (SLA, cm\(^{2}\) g\(^{-1}\)), and leaf mass ratio (LMR, g g\(^{-1}\)) in determining relative growth rate (RGR, g g\(^{-1}\) day\(^{-1}\)) measured from flowering to seed-filling stage.

<table>
<thead>
<tr>
<th>CO(_2)</th>
<th>Water</th>
<th>NA (g cm(^{-2}) day(^{-1}))</th>
<th>SLA (cm(^{2}) g(^{-1}))</th>
<th>LMR (g g(^{-1}))</th>
<th>RGR (mg g(^{-1}) day(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient</td>
<td>normal</td>
<td>0.41bc</td>
<td>229.17a</td>
<td>0.39a</td>
<td>36.67a</td>
</tr>
<tr>
<td></td>
<td>drought</td>
<td>0.34</td>
<td>150.83b</td>
<td>0.27c</td>
<td>14.19c</td>
</tr>
<tr>
<td>Elevated</td>
<td>normal</td>
<td>0.57a</td>
<td>217.12a</td>
<td>0.32b</td>
<td>39.87a</td>
</tr>
<tr>
<td></td>
<td>drought</td>
<td>0.45b</td>
<td>162.48b</td>
<td>0.31bc</td>
<td>22.08b</td>
</tr>
<tr>
<td>ANOVA CO(_2) (C)</td>
<td>ns</td>
<td>**</td>
<td>ns</td>
<td>ns</td>
<td>**</td>
</tr>
<tr>
<td>Water (W)</td>
<td>*</td>
<td>***</td>
<td>*</td>
<td>ns</td>
<td>***</td>
</tr>
<tr>
<td>C*W</td>
<td>**</td>
<td>***</td>
<td>*</td>
<td>ns</td>
<td>***</td>
</tr>
</tbody>
</table>

Values followed by the same letter within each genotype are not significantly different at \(P>0.05\) as determined by the Tukey’s mean comparison test. ns: not significant at \(P>0.05\). * Significantly different at \(P<0.05\). ** Significantly different at \(P<0.01\). *** Significantly different at \(P<0.001\).

Elevated CO\(_2\) improves WP and Pn in response to drought stress:

Drought markedly reduced WP by 15.5% in atmospheric CO\(_2\) concentration and by 7.2% in elevated CO\(_2\) concentration (Fig. 2). Elevated CO\(_2\) significantly alleviated drought-induced decrease in WP, which is consistent with the former point. However, elevated CO\(_2\) alone showed no significant effect on WP. This finding may be attributed to the different growth stages and nutrition situations of asynchronous growth. Vapor pressure deficit among treatments showed no obvious differences on average across all the reproductive growth stages (Fig. 2). Meta-analysis indicated that elevated CO\(_2\) did not alter the response of stomatal conductance to vapor pressure deficit, soil water content, or atmospheric condition.

Elevated CO\(_2\) alone significantly increased Pn by 8.9% on average across the reproductive growth stages (Fig. 3). This finding may be related to the carboxylation rate of rubisco and nitrogen content. Drought dramatically decreased the value significantly by 23.2% and 8.3% under ambient and elevated CO\(_2\) concentrations, respectively. The response to the combination treatment of elevated CO\(_2\) and drought was improved significantly compared with that to the drought treatment alone. A recent report has shown that photosynthesis is positively correlated with seed yield.

Under ambient and elevated CO\(_2\) concentrations, drought significantly decreased chlorophyll content by 11.4 and 7.3%, respectively, and carotenoid content by 16.5 and 15.7%, respectively. Nonetheless, no significant changes in chlorophyll and carotenoid contents were observed between the two CO\(_2\) concentrations irrespective of water condition. Although the drought-induced decrease in chlorophyll a/b was related to weakened thylakoids stacking, no significant differences were
found among all treatments on average across the reproductive growth stages. This finding is similar to the response of quinoa to drought. Elevated CO2 increased Pn but did not significantly change photosynthetic pigment content. This finding may be attributed to the mesophyll diffusion of additional carbon and kinetics of the dark reaction.

Total soluble sugar and soluble protein contents: To further analyze the physiological effects of long-term elevated CO2 and drought conditions, we observed antioxidant activities associated with the osmotic regulation pathway of soybean leaves. The total soluble sugar content of soybean under the four treatments during all measuring stages showed an increasing trend (Fig. 4). Drought significantly increased the soluble sugar content under enriched CO2 at 44 and 60 days after planting (DAP). However, elevated CO2 treatment alone markedly increased soluble sugar content by 21.5% on average, which is consistent with former outcomes. This finding may be attributed to higher photosynthetic capacity. Compared with drought alone, the combination treatment of elevated CO2 and drought increased soluble sugar content by 20.5% on average across all the reproductive growth stages. These findings suggested that CO2 enrichment enhanced the photoassimilation and increased the partitioning of carbon to nodules in soybean under drought condition.

Severe water stress disturbed protein metabolism. The present study demonstrated that soluble protein content showed a descending trend across all the measuring stages. Elevated CO2 increased soluble protein content by 3.1 and 8.3% on average under well-irrigated and drought conditions (Fig. 4). Elevated CO2 showed no significant effects on leaf protein content at 73DAP, which is the latter part of the growth season.

Antioxidant enzyme activity and MDA content: As shown in Table 2, the total SOD, Cu-Zn SOD, and POD activities of soybean leaves significantly increased in response to drought under ambient CO2 concentration. In addition, the interaction between elevated CO2 and drought on POD activity significantly decreased. However, total SOD and Cu-Zn SOD activities did not significantly change. As growth progressed, total SOD activity decreased and Cu-Zn SOD activity increased, indicating their important roles in regulating reactive oxygen species (data not shown). Qiu et al. pointed out that elevated CO2 can increase oxidative stress in plants. The present results showed that elevated CO2 slightly increased antioxidant enzyme activity without significant difference. Moreover, elevated CO2 concentration alone had no significant effect on POD activity as previously reported by Lambreva et al. By contrast, Pritchard et al. found that elevated
CO₂ reduces the activity of ascorbic acid peroxidase and other antioxidant enzymes in two soybean genotypes. For cereals, the enzyme responses to enhanced CO₂ level were found to vary with different plant species and phenophases 40.

Drought significantly increased the MDA content, which is in agreement with the results of previous studies 45. However, the MDA content of plants grown under the combination treatment of drought and enriched CO₂ was still significantly higher than that of plants grown under ambient CO₂ (Table 2). This finding suggested that elevated CO₂ could not recover the membrane damage caused by drought 44. Zhao et al. 46 revealed that the beneficial effects of elevated CO₂ compensate for the negative effects imposed by O₂ stress, with the latter partly counteracting the positive effects of the former. In general, the effects of the combination treatment of elevated CO₂ and drought on plant growth are complex, and not a simple superposition of breaking and/or compensating effects of drought alone.

### Table 2. Super oxygen dehydrogenises (totle-SOD), (Cu-Zn SOD), peroxidase activity (POD), and malonaldehyde content (MDA) measured from flowering to seed-filling stage.

<table>
<thead>
<tr>
<th>CO₂</th>
<th>Water</th>
<th>T-SOD (U/mg protein)</th>
<th>Cu-Zn SOD (U/mg protein)</th>
<th>POD (U/mg protein)</th>
<th>MDA (mg/g FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ambient</td>
<td>normal</td>
<td>38.27b</td>
<td>43.27b</td>
<td>18.19b</td>
<td>15.06c</td>
</tr>
<tr>
<td></td>
<td>drought</td>
<td>42.36a</td>
<td>49.57a</td>
<td>20.49a</td>
<td>17.40ab</td>
</tr>
<tr>
<td>elevated</td>
<td>normal</td>
<td>39.34ab</td>
<td>46.46ab</td>
<td>18.33b</td>
<td>16.65bc</td>
</tr>
<tr>
<td></td>
<td>drought</td>
<td>37.85b</td>
<td>44.06b</td>
<td>17.13c</td>
<td>19.01a</td>
</tr>
</tbody>
</table>

ANOVA CO₂ H₂O ns ns *** *

| CO₂ + H₂O | ns | ns | *** |

Values followed by the same letter within each genotype are not significantly different at P < 0.05 as determined by the Tukey’s means comparison test. ns: not significant at P = 0.05. * Significantly different at P < 0.05. ** Significantly different at P < 0.01. *** Significantly different at P < 0.001.

### Conclusions

This study confirmed the hypothesis that elevated CO₂ concentration alone has a beneficial effect on Pn, WP, NAR, RGR, and total soluble sugar content across the growing stage under normal water and drought conditions. However, its effect on photosynthetic pigments remains unclear. The combination treatment of elevated CO₂ and drought alleviated the adverse effects of drought on WP, Pn, NAR, RGR, and soluble sugar content of leaves. However, under drought stress, enhanced CO₂ concentration did not stimulate antioxidant enzyme activity significantly and did not alleviate membrane lipid peroxidation effects. It was possible that enzyme responses to enhanced CO₂ vary with the plant species and different phenophases. The intricate mechanism still needs more data to be interpreted.

### Acknowledgements

This work was supported by the National Natural Science Foundation of China (31230050; 30971797; 31170415; 3100191; 30870411), Key Project in the National Science & Technology Pillar Program of China (2012BAD008B02), Knowledge Innovation Program of the Chinese Academy of Sciences (KSCX2-EW-Q-25), Natural Science Foundation of Hebei Province (C2011503003; C2012503003).

### References


