Increased CO₂ concentrations increasing water use efficiency and improvement PSII function of mulberry seedling leaves under drought stress

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ABSTRACT
In this paper, the effects of different CO₂ concentrations (400 and 800 μmol·mol⁻¹) on photosynthetic gas exchange and chlorophyll fluorescence of mulberry (Morus alba L.) seedling leaves under drought stress were studied using an artificial climate chamber. The results showed that under non-drought conditions, the stomatal concentrations (Gs) and transpiration rate (Tr) of mulberry seedling leaves decreased slightly with increased CO₂ concentrations, but the net photosynthetic rate (Pn) increased significantly. Under mild drought stress (5 days of drought), higher CO₂ concentrations significantly affected the photosynthetic gas exchange of mulberry seedling leaves, but had little effect on chlorophyll fluorescence. Under severe drought stress (10 days of drought), increased CO₂ concentrations not only alleviated drought stress by increasing the WUE of mulberry seedling leaves, but also significantly increased its PSII photochemical activity, which promoted electron transfer on the PSII acceptor side. In conclusion, increased CO₂ concentrations could raise the WUE of mulberry seedling leaves under normal water conditions and drought stress, and this effect was more significant under drought stress. Under severe drought stress, increased CO₂ concentrations improved the drought resistance of mulberry seedlings by improving their PSII function.

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Introduction
The rising concentration of CO₂ in the atmosphere is considered to be one of the main driving forces of global climate change. Studies have shown that CO₂ concentrations increased from 280 μmol·mol⁻¹ in the industrial revolution to 388 μmol·mol⁻¹ today, and are expected to increase to 700 μmol·mol⁻¹ by the end of the twenty-first century. Global warming caused by increased CO₂ concentrations will have a profound impact on the environment and climate change (Fangmeier et al. 2000; Aranjuelo et al. 2011; Yin et al. 2017). As a substrate for plant photosynthesis, higher CO₂ concentrations will affect plant photosynthesis and their physiological and biochemical processes (Prasad et al. 2009), by increasing intercellular CO₂ and carboxylation efficiency, reducing photorespiration (Bowes 2003; Ainsworth and Long 2005; Zhang and Dang 2005; Stiling et al. 2013), increasing WUE (Fleisher et al. 2008; Pazzaglia et al. 2016; Varga et al. 2017), increasing the content of soluble sugar and starch (Bindi et al. 2001; Sun et al. 2012) and enhancing the activity of antioxidant enzymes (SOD and CAT) (Marabottini et al. 2001; Schwanz and Polle 2001) etc. Other researchers have reported that increased CO₂ concentrations could lead to a decrease in stomatal density (Xu et al. 2017) and conductance (Ainsworth and Long 2005), the reduction of partial pressure of oxygen in cells, and a decrease in plant leaf respiration rate (Gifford 1995; Ziska and Bunce 1998). Rising CO₂ promotes plant photosynthesis and growth and increases crop yield (Murray et al. 2000; Li et al. 2007), but other studies have demonstrated that high CO₂ concentrations change the source sink balance of crops and affect the operation of organic matter from the vegetative organ to grain (Biswas et al. 2013; Bourgault et al. 2013). The decreased transpiration rate caused by reduced stomatal conductance indirectly limits nutrition uptake for plants, affecting the growth and quality of crops (Loladze 2002; Högy and Fangmeier 2008; Högy et al. 2009; Myers et al. 2014; Li et al. 2016; Dong et al. 2018).

Global climate change caused by the increase in CO₂ concentrations causes extreme weather, such as drought, and these jointly affect the physiological and ecological processes of plants (Jiang et al. 2016; Xu et al. 2016). Drought stress is one of the important limiting factors for agricultural production and leads to a decline in crop yield and quality (Ye et al. 2018; Zhang et al. 2019). Plant photosynthesis is a process that is sensitive to drought. Under drought stress, wilting and curling of plant leaves and reduced chlorophyll content occur frequently. More specifically, drought stress could suppress a series of physiological processes, such as the decrease or closure of stomatal conductance, the inhibition of chlorophyll synthesis or the acceleration of degradation, the reduction of photosynthetic enzyme activity, and reduced photosynthetic carbon assimilation (Pukacki and Kamińska-Rożek 2005; Nikolaeva et al. 2010; Albert et al. 2011) etc. Moreover, drought stress also results in the reduction of PSII and PSI activities, the resistance of...
photosynthetic electron transfer and the accumulation of excess energy (Schansker et al. 2005; Zhang et al. 2018). Excess electrons and energy may lead to the increase of reactive oxygen species (ROS) content, and excessive ROS breaks the balance of redox in plants and causes peroxidation membrane damage (Gill and Tuteja 2010; Wang et al. 2017).

Many studies have shown that increased CO2 concentrations can alleviate the inhibition of abiotic stress on plant growth and physiological function (Shanmugam et al. 2013; Medina et al. 2016; Li et al. 2017; Wei et al. 2018). This increase causes a rise of intercellular CO2 concentration ($C_i$), which could compensate for the CO2 restriction caused by the decrease of stomatal conductance under stress (Morrison and Gifford 1983). It also reduces transpiration by reducing the stomatal conductance ($G_s$), thus improving the WUE of plant leaves under stress (Fleisher et al. 2008; Pazzaglia et al. 2016; Varga et al. 2017). Rising CO2 increases carbohydrate concentration under drought stress, resulting in the enhancement of cell osmotic potential and drought resistance of plants (Dong et al. 2015). The leaves of mulberry trees (Morus alba L.) can not only be used as silkworm feed, but also have antioxidants with important medicinal value (Katsube et al. 2006). In addition, mulberry has strong drought resistance and is an excellent tree species for sand fixation and water and soil conservation, which has important economic and ecological value (Sarkar et al. 2017). However, there are no reports on the photosynthetic functions of mulberry seedling leaves under drought stress with increased CO2 concentrations, all the seedlings were cultured in the same conditions, and most of the studies on plant photosynthesis have focused on the stoma and photosynthetic gas exchange. There are few studies of the PSII function of plant light reactions under this condition. Therefore, under the condition of natural drought, the effects of rising CO2 on photosynthetic gas exchange of mulberry seedling leaves, instantaneous WUE, and PSII function were studied in this paper to provide some basic data for the mechanism of plant photosynthesis under drought stress.

Materials and methods

Test materials and treatment

The experiment was conducted at the Water Conservancy Institute of Northeast Agricultural University in 2018. The tested tree species, mulberry (Morus alba L.), is a local mulberry variety known as ‘Qinglong mulberry’ in Heilongjiang province. The seeds were provided by the Sericulture Institute in Heilongjiang province and test materials were one-year mulberry seedlings with a height of about 15 cm. Every two seedlings were planted in a pot (30 cm in diameter, 28 cm in height) filled with fully mixed substrates of peat and perlite with a volume ratio of 1:1.

Ten pots of mulberry seedlings with relatively uniform growth were selected as experimental materials and were evenly placed in artificial climate box (Conviron E8, Canada) with CO2 concentrations of 400 and 800 μmol·mol$^{-1}$. The artificial climate box is equipped with an automatic control system of CO2 concentration, which is connected with the external CO2 cylinder. Aside from the different CO2 concentrations, all the seedlings were cultured in the same conditions with a temperature of 25°C, light intensity of 1000 μmol·m$^{-2}·s^{-1}$, a photoperiod of 12/12 (light/dark), and relative humidity of 75%. Sufficient water was added to the mulberry seedlings to reach the saturated relative water content in soil before drought treatment. Finally, the natural drought treatment was carried out, and photosynthetic gas exchange and chlorophyll fluorescence were measured at days 0, 5, and 10.

Determination of parameters

Determination of the parameters of photosynthetic gas exchange

The reciprocal second pieces of completely unfurled mulberry seedling leaves on different drought days were selected. The net photosynthetic rate ($P_n$), stomatal conductance ($G_s$), transpiration rate ($T_r$), and intercellular CO2 concentration ($C_i$) were measured at 9:00 am using Ciras-2 Portable Photosynthesis instrument (Hansatch, UK). The air temperature, air relative humidity, CO2 concentration in the atmospheric and PFD during measurement were maintained as 25°C, 60 ± 3% relative humidity, 380 μmol·mol$^{-1}$ and 1000 μmol·m$^{-2}·s^{-1}$, respectively, with an automatic leaf cuvette of the Ciras-2. The leaves were enclosed in the leaf cuvette for 60 s before measure. and the instantaneous water use efficiency (WUE) of the leaves was calculated, where WUE = $P_n/T_r$. The test was repeated five times.

Chlorophyll content and nonphotochemical quenching (NPQ)

Chlorophyll content was determined using CCM-200 chlorophyll meter (Opti-Sciences, USA), and the SPAD readings of chlorophyll meter were used as relative leaf chlorophyll contents.

After 30 min dark adaptation, the leaves of mulberry seedlings were measured by FMS-2 (Hansatch Company, UK) instrument with saturated pulsed light of 8000 μmol·m$^{-2}·s^{-1}$, and the maximum fluorescence (Fm) was determined. The leaves of mulberry seedlings under light adaptation were irradiated with 400 μmol·m$^{-2}·s^{-1}$ for 30 s, then saturated pulsed light was turned on for 0.7 s. The maximum fluorescence (Fm’) under light adaptation was measured, and non-photochemical quenching (NPQ), NPQ = (Fm−Fm’)/Fm’ (Xu et al. 2018) were calculated.

Determination of fast chlorophyll fluorescence induction curve (OJIP)

The reciprocal second pieces of completely unfurled mulberry seedling leaves after different treatments were selected. After 30 min of dark adaptation with dark adaption clips, the OJIP curves for the treated leaves were measured using a Multi-Function Plant Efficiency Analyzer (Handy-PEA) from Hansatch, and five repeated tests were conducted for each treatment. The OJIP curves were plotted with the average of the five repetitions, in which O, J, I and P points corresponded to the time points of 0.01, 2, 30 and 1000 ms, respectively. Their respective relative fluorescence intensity was expressed as $F_0$, $F_t$, $F_i$ and $F_p$. The OJIP curves were normalized according to formulas $V_{O-K} = (F_t−F_0)/(F_p−F_0)$, $V_{O-J} = (F_t−F_O)/(F_t−F_O)$, and $V_{O-J}$ of the relative variable fluorescence $V_t$ at the J point (2 ms) of the $V_{O-K}$ curve, the $V_K$ at the K point (0.3 ms) of the $V_{O-J}$ curve, and the $V_L$ at the L point (0.15 ms) of the $V_{O-K}$ curve were obtained by this formula, in which $F_t$ was the relative fluorescence intensity at each time point on the OJIP curve. The values on the standardized
V_{O,P}, V_{O,J} and V_{O,K} curves of mulberry seedling leaves under different drought days were compared with CK values, which were expressed as \( \Delta V_{O,P}, \Delta V_{O,J} \) and \( \Delta V_{O,K} \).

A JIP-test analysis was conducted on the OJIP curve to obtain the maximum photochemical efficiency of PSII (\( F_{v}/F_{m} \)), the performance index of PSII based on absorption (\( PI_{ABS} \)), and the performance index of electron flux to the final PSI electron acceptors. Both PSI and PSII (\( PI_{total} \)), absorption flux per reaction center (\( ABS/RC \)), electron transport flux per reaction center (\( ET_{r}/RC \)), dissipated energy flux per reaction center (\( DI_{r}/RC \)), absorption flux per excited cross section (\( ABS/CS_{m} \)), electron transport flux per excited cross section (\( ET_{r}/CS_{m} \)) and dissipated energy flux per reaction center per excited cross section (\( DI_{r}/CS_{m} \)) and the number of the active response centers per unit area (\( RC/CS_{m} \)). JIP-test analysis of OJIP curves by the method of Strasser et al. (1995).

**Data analysis**

Excel (2003) and SPSS (22.0) software were used for statistical analysis. One-way ANOVA and least significant difference (LSD) tests were used to compare the differences between different data sets.

**Results and analysis**

**Parameters of photosynthetic gas exchange**

As shown in Figure 1, on day 0 of drought the values of \( \Gamma \) and \( T_{r} \) for mulberry seedling leaves at a CO\(_2\) concentration of 800 \( \mu \)mol·mol\(^{-1}\) were slightly lower than under a CO\(_2\) concentration of 400 \( \mu \)mol·mol\(^{-1}\). The value of \( P_{n} \) increased by 26.85% (\( P < 0.01 \)) and, WUE also increased significantly. With longer drought time, the values of \( P_{n} \), \( \Gamma \) and \( T_{r} \) for mulberry seedling leaves were greatly reduced. Although there was a significant difference in \( T_{r} \) between CO\(_2\) concentrations of 400 and 800 \( \mu \)mol·mol\(^{-1}\) on the 5th drought day, there was no obvious difference between \( \Gamma \) and \( T_{r} \) at various CO\(_2\) concentrations. On the 5th day of drought, \( \Gamma \) of mulberry seedling leaves decreased significantly with that on the 0th day, but increased on the 10th day of drought compared with that on the 5th day. However, different CO\(_2\) concentrations had no significant effect on \( \Gamma \) of Mulberry Seedling Leaves under drought stress. The values of \( P_{n} \) and WUE at a CO\(_2\) concentration of 800 \( \mu \)mol·mol\(^{-1}\) were significantly higher than those under 400 \( \mu \)mol·mol\(^{-1}\). The increase of \( \Gamma \) and WUE at a CO\(_2\) concentration of 800 \( \mu \)mol·mol\(^{-1}\) was significantly greater than that of 400 \( \mu \)mol·mol\(^{-1}\) treatment.

**OJIP curves and the relative fluorescence intensity of characteristic points (O and P)**

Figure 2(a,b) show that the change in the OJIP curves of mulberry seedling leaves under CO\(_2\) concentrations of 400 and 800 \( \mu \)mol·mol\(^{-1}\) on the 5th drought day were smaller than on drought day 0. On the 10th drought day, the relative fluorescence intensity \( F_{o} \) of point O on the OJIP curve at CO\(_2\) concentration of 400 \( \mu \)mol·mol\(^{-1}\) increased, while the relative fluorescence intensity \( F_{m} \) decreased significantly (Figure 2(a)). In addition, the change in the OJIP curve at 800 \( \mu \)mol·mol\(^{-1}\) was significantly less than at 400 \( \mu \)mol·mol\(^{-1}\) on the 10th drought day (Figure 2(b)).

Quantitative analysis of \( F_{o} \) and \( F_{m} \) in Figure 3 showed that under a CO\(_2\) concentration of 400 \( \mu \)mol·mol\(^{-1}\) the \( F_{o} \) of mulberry seedling leaves increased by 12.70% (\( P < 0.01 \)) at the 10th drought day compared with drought day 0, while \( F_{m} \) decreased by 6.92% (\( P > 0.05 \)). At a CO\(_2\) concentration of 800 \( \mu \)mol·mol\(^{-1}\), the changes in \( F_{o} \) and \( F_{m} \) were both less than these under 400 \( \mu \)mol·mol\(^{-1}\). After 10 days of drought, the value of \( F_{o} \) at a CO\(_2\) concentration of 800 \( \mu \)mol·mol\(^{-1}\) was 5.48% (\( P > 0.05 \)) less than at 400 \( \mu \)mol·mol\(^{-1}\), while the \( F_{m} \) was 5.95% (\( P > 0.05 \)) higher.

**PSII photochemical efficiency**

After 10 days of drought there was no significant difference in \( F_{v}/F_{m}, PI_{ABS} \) or \( PI_{total} \) of mulberry seedling leaves under CO\(_2\) concentrations of 400 and 800 \( \mu \)mol·mol\(^{-1}\). With longer drought time, the \( F_{v}/F_{m}, PI_{ABS} \) and \( PI_{total} \) values decreased, but the change was not clear at drought day 5 and there was no significant difference between the treatments of 400 and 800 \( \mu \)mol·mol\(^{-1}\). On the 10th drought day, the \( F_{v}/F_{m}, PI_{ABS} \) and \( PI_{total} \) values at CO\(_2\) concentrations of 800 \( \mu \)mol·mol\(^{-1}\) were respectively 2.78% (\( P < 0.05 \)), 35.13% (\( P < 0.01 \)) and 80.61% (\( P < 0.01 \)) higher than those at 400 \( \mu \)mol·mol\(^{-1}\), and the difference in \( PI_{ABS} \) and \( PI_{total} \) was significantly greater than that of \( F_{v}/F_{m} \) (Figure 4).

**Standardization of the O-P curve and relative variable fluorescence \( V_{j} \) at point J (2 ms)**

The relative fluorescence intensity at point O on the OJIP curve for mulberry seedling leaves was defined as 0 and at point P was defined as one across all treatments to standardize the OJIP curve. Compared to drought day 0 (Figure 5(a,b)), the relative variable fluorescence \( V_{j} \) of J point (2 ms) of the standardized O-P curve at the 5th and 10th drought days increased in varying degrees, and the increase of \( V_{j} \) at the 10th day was greater than at the 5th day.

Quantitative analysis of \( V_{j} \) shown in Figure 6 indicated that there was no significant difference in \( V_{j} \) of mulberry seedling leaves under CO\(_2\) concentrations of 400 and 800 \( \mu \)mol·mol\(^{-1}\) at days 0 and 5 of drought. When the drought time was 10 days, the value of \( V_{j} \) increased significantly compared with drought day 0, but the increase in \( V_{j} \) at a CO\(_2\) concentration of 800 \( \mu \)mol·mol\(^{-1}\) was less than at 400 \( \mu \)mol·mol\(^{-1}\).

**Standardization of the O-J curve and relative variable fluorescence \( V_{k} \) at point K (0.3 ms)**

To further analyze the change in relative variable fluorescence at point K at 0.3 ms, the relative fluorescence intensities were set as above (2.5) and the O-J curve was standardized. After standardization, the relative variable fluorescence \( V_{k} \) at 0.3 ms on the standardized O-J curves under different treatments was significantly different (Figure 7(a,b)). The \( V_{k} \) of mulberry seedling leaves increased under drought stress. The increase at the 10th drought day was significantly greater than that at the 5th day, and the increase of \( V_{k} \) under a CO\(_2\) concentration of 400 \( \mu \)mol·mol\(^{-1}\) was significantly greater than under 800 \( \mu \)mol·mol\(^{-1}\).

As shown in Figure 8, quantitative analysis of the changes in \( V_{k} \) showed that there was no significant difference in \( V_{k} \) of
Figure 1. Effects of increased CO$_2$ concentrations on net photosynthetic rate (a), stomatal conductance (b), transpiration rate (c), intercellular CO$_2$ concentration value (d) and water use efficiency (e) of mulberry seedling leaves under drought stress. Note: Lower case letters indicate significant differences ($P < 0.05$) and capital letters indicate highly significant differences ($P < 0.01$). The CO$_2$ concentrations of 400 and 800 $\mu$mol·mol$^{-1}$ are represented as 400 and 800, respectively.

Figure 2. Effects of increased CO$_2$ concentrations on OJIP curves of mulberry seedling leaves under drought stress.
mulberry seedling leaves under CO2 concentrations of 400 and 800 \(\mu\text{mol} \cdot \text{mol}^{-1}\) at the 0 and 5 days of drought. At the 10th drought day, the value of \(V_K\) under a CO2 concentration of 800 \(\mu\text{mol} \cdot \text{mol}^{-1}\) was 7.46\% \((P < 0.05)\) lower than that under 400 \(\mu\text{mol} \cdot \text{mol}^{-1}\).

In order to further analyze the change of relative variable fluorescence \(V_L\) at point L (0.15 ms), the relative fluorescence intensities were set as above (2.5) and the O-K curve was standardized. After standardization, the \(V_L\) of mulberry seedling leaves under a CO2 concentration of 400 \(\mu\text{mol} \cdot \text{mol}^{-1}\) increased significantly, while the change under 800 \(\mu\text{mol} \cdot \text{mol}^{-1}\) was small (Figure 9(a,b)).

**Standardization of the O-K curve and relative variable fluorescence \(V_L\) at point L (0.15 ms)**

In order to further analyze the change of relative variable fluorescence \(V_L\) of L point at 0.15 ms, the relative fluorescence intensities were set as above (2.5) and the O-K curve was standardized. After standardization, the \(V_L\) of mulberry seedling leaves under a CO2 concentration of 400 \(\mu\text{mol} \cdot \text{mol}^{-1}\) increased significantly, while the change under 800 \(\mu\text{mol} \cdot \text{mol}^{-1}\) was small (Figure 9(a,b)).
Quantitative analysis of $V_L$ (Figure 10) showed that under a CO$_2$ concentration of 400 μmol·mol$^{-1}$, the $V_L$ of mulberry seedling leaves at the 5th and 10th drought day increased by 3.41% ($P > 0.05$) and 9.26% ($P < 0.01$), respectively, compared with drought day 0. However, there was no significant difference of $V_L$ observed on drought days 0, 5, and 10 under 800 μmol·mol$^{-1}$.

**Unit reaction center and energy distribution parameters on unit area**

As shown in Figure 11(a), at a CO$_2$ concentration of 400 μmol·mol$^{-1}$, there were no significant difference in each unit reaction center and the energy distribution parameters of unit cross-sectional area on days 0 and 5. By the 10th drought day, however, the ABS/RC, DI$_o$/RC, and DI$_o$/CS$_m$ ratios increased by 13.59% ($P < 0.01$), 39.56% ($P < 0.01$), and 14.11% ($P < 0.05$), respectively, compared to day 0. There was no significant difference on ET$_o$/RC and ABS/CS$_m$ but the ET$_o$/CS$_m$ decreased by 19.86% ($P < 0.01$). At a CO$_2$ concentration of 800 μmol·mol$^{-1}$ (Figure 11(b)), the energy distribution variability of mulberry seedling leaves was similar to that at 400 μmol·mol$^{-1}$ but the changes were significantly less and the differences between each energy distribution parameter on drought days 0, 5 and 10 were insignificant.

**Changes in RC/CS$_m$ in the active reaction center of unit area (Figure 12)** indicated that there was no significant difference in RC/CS$_m$ in mulberry seedling leaves at CO$_2$ concentrations of 400 and 800 μmol·mol$^{-1}$ between drought days 0 and 5. At the 10th drought day, however, the value of RC/CS$_m$ decreased by 17.98% ($P < 0.01$) at 400 μmol·mol$^{-1}$ compared to drought day 0, and there was no significant reduction at 800 μmol·mol$^{-1}$.

**SPAD value and NPQ**

It can be seen that the SPAD and NPQ of mulberry seedling leaves did not change significantly on the 5th and 0th day of drought; however, the SPAD value and NPQ showed an increase at the 10th drought day under high CO$_2$ concentration conditions.

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**Figure 7.** Effects of increased CO$_2$ concentrations on $V_{O, J}$ and $\Delta V_{O, J}$ curves of mulberry seedling leaves under drought stress.

**Figure 8.** Effects of increased CO$_2$ concentrations on $V_K$ of mulberry seedling leaves under drought stress.

**Figure 9.** Effects of increased CO$_2$ concentrations on $V_{O,K}$ and $\Delta V_{O,K}$ curves of mulberry seedling leaves under drought stress.

**Figure 10.** Effects of increased CO$_2$ concentrations on $V_L$ of mulberry seedling leaves under drought stress. The CO$_2$ concentrations of 400 and 800 μmol·mol$^{-1}$ are represented as 400 and 800, respectively.
drought (Figure 13), but on the 10th day of drought, the PAD value of mulberry seedling leaves decreased, while NPQ increased significantly. The SPAD value of mulberry seedling leaves with CO₂ concentration of 800 μmol·mol⁻¹ was slightly lower than that of 400 μmol·mol⁻¹, but the difference was not significant. The NPQ of mulberry seedling leaves with CO₂ concentration of 800 μmol·mol⁻¹ was lower than that of 400 μmol·mol⁻¹ CO₂ concentration of 13.65% (P < 0.05).

### Discussion

Some studies have suggested that increased CO₂ concentrations cause a decrease Gₛ and the partial closure of the stomata (Murray 1995; Kang et al. 2002; Alejandro et al. 2005; Ainsworth and Rogers 2007), and Curtis et al. also demonstrated that doubled CO₂ reduces the average stomatal conductance by about 11% (Curtis and Wang 1998). Increased CO₂ is reported to decrease the stomatal density of winter wheat (Woodward and Kelly 2010). In this experiment, on drought day 0, the Gₛ and Tᵣ of mulberry seedling leaves under CO₂ concentrations of 800 μmol·mol⁻¹ were lower than those under 400 μmol·mol⁻¹, but the significant increase in Pᵦ indicated that Gₛ was not a limiting factor for Pᵦ enhancement under doubled CO₂. The increase in CO₂ concentration compensated for the decrease of CO₂ caused by Gₛ reduction, thus promoting photosynthesis. In addition, the increase in CO₂ can improve the activity of the Rubisco enzyme and its carboxylation (Li et al. 2011). It can also increases photosynthetic capacity by increasing the substrate and inhibition of photorespiration (Farquhar et al. 1980; Chen et al. 2005) which is in line with the results of Peng et al. (2004) that higher CO₂ can increase photosynthetic rate of plants.

Drought stress can increase plant ABA content (And and Zhang 2003; Moretti et al. 2010; Sun et al. 2014), which increases WUE by inducing stomatal closure to adapt to...
drought stress (Liu et al. 2005). As the substrate of photosynthesis and the stomatal regulator, increased CO₂ concentrations cause a decrease in Gᵣ, but the effect on CO₂ absorption is far less than on water vapor diffusion. Therefore, increased CO₂ reduces Gᵣ but alleviates drought stress by reducing transpiration loss and increasing WUE (Morison and Gifford 1983; Wu and Wang 2000; Ainsworth and Rogers 2007; Pazzaglia et al. 2016). The results of Hamerlynck et al. (2000) revealed that there was a significant interaction between the effects of increased CO₂ and drought stress on Pᵣ and Gᵣ, and high CO₂ could improve the adaptability of plants to drought. Similar results were also obtained in this experiment: under drought stress, Pᵣ and Tᵣ were reduced along with the decreasing Gᵣ, and the decrease in Tᵣ was significantly greater than that of Pᵣ, which resulted in the increase of WUE. In the different drought day treatments, the WUE of mulberry seedling leaves under a CO₂ concentration of 800 μmol·mol⁻¹ was significantly higher than under 400 μmol·mol⁻¹, indicating that the increased CO₂ enhanced the adaptability to drought stress by increasing WUE. CO₂ treatment at 800 μmol·mol⁻¹ concentration did not significantly increase the Gᵣ of mulberry seedling leaves, which may be related to the decrease of Gᵣ in mulberry seedling leaves due to the increase of CO₂ concentration. In addition, Gᵣ in detail leaves of mulberry seedlings decreased significantly with the decrease of Gᵣ on the 5th day of drought, but increased on the 10th day of drought compared with the 5th day, indicating that the decrease of Pᵣ in mulberry seedlings on the 10th day of drought might be limited by non-stomatal factors.

Under drought stress, the dark reaction on plant leaves is inhibited, and the accumulation of assimilation (ATP and NADPH) can inhibit the process of light reaction and reduce the activity of the PSI reaction center (Liu et al. 2006). In this experiment, as drought time increased the Fᵢ/Fₘₒ PI_ABS and PI_TOTAL values of mulberry seedling leaves decreased, especially PI_ABS and PI_TOTAL. This indicated that the photochemical activity of PSI was reduced under drought stress. One study reported that increased CO₂ affects the PSI structure of plant leaves, thus affecting photosynthetic energy conversion and electron transfer (Xu et al. 2016). However, the effect of an increase in CO₂ on Fᵢ/Fₘₒ PI_ABS and PI_TOTAL of mulberry seedling leaves was not significant on drought days 0 and 5 in this experiment. At the 10th drought day, the Fᵢ/Fₘₒ PI_ABS and PI_TOTAL under a CO₂ concentration of 800 μmol·mol⁻¹ were significantly higher than under 400 μmol·mol⁻¹. This result was similar to the findings of Li et al. (2008), that rising CO₂ concentrations improve the maximum quantum yield of PSII and photochemical quenching coefficient and decrease the non-photochemical quenching coefficient on cucumber seedling leaves under drought stress, which reduces the damage of drought stress to some extent. The decrease of SPAD value of chlorophyll content was also slightly lower than that of 400 μmol·mol⁻¹, indicating that increased CO₂ could alleviate degradation of chlorophyll under severe drought stress.

Under stress, the blocking sites of photosynthetic electron transfer in plants are normally located on the donor side and acceptor side of the PSI reaction center (Jiang et al. 2008; Shuang et al. 2009). It was found that the main reason why increased CO₂ alleviates photosynthetic restriction of plant leaves was related to the promotion of photosynthetic electron transport (Zhu et al. 2018). In order to analyze the effect of increased CO₂ on photosynthetic electron transfer in mulberry seedling leaves under drought stress, the OJIP curves were standardized by O-P and O-J, where the relative variable fluorescence Vᵣ at point J at 2 ms on the standardized O-P curve indicated that electrical transmission from Qₓ and Qₓ in photosynthetic electron transfer chain was hindered (Zhang et al. 2017; Xu et al. 2018). The increase in relative variable fluorescence Vᵣ at point K at 0.3 ms on the standardized O-J curve was considered to be a specific marker for the loss of OEC activity on the PS II electron donor side (Zhang et al. 2012, 2016). In this experiment, the Vᵣ and Vₓ of mulberry seedlings increased to varying degrees under drought stress, indicating that the cause of PSII photochemical activity degradation under drought stress was related to the obstruction of electron transport on the PSI donor side and acceptor side. On drought days 0 and 5, there was no significant difference between Vᵣ and Vₓ at CO₂ concentrations of 400 and 800 μmol·mol⁻¹. On the 10th day of drought, Vᵣ and Vₓ at a CO₂ concentration of 800 μmol·mol⁻¹ were significantly lower than at 400 μmol·mol⁻¹, indicating that the increase in CO₂ alleviated the damage of OEC in mulberry seedling leaves under severe drought stress and promoted electron transfer from Qₓ to Qₓ on the PSI acceptor side. These results are in agreement with Cui (2017) that an increase in CO₂ improves the abundance of psbL, PSI chlorophyll a-b binding protein, and the protein subunit in the PSI reaction center of cucumber seedling leaves under drought stress, enhancing electron transfer rate. In addition, the Vᵣ of mulberry seedling leaves under a CO₂ concentration of 800 μmol·mol⁻¹ at the 10th day of drought was significantly less than under 400 μmol·mol⁻¹. The increase in Vᵣ was an important sign of the changes in the fluidity of thylakoid membrane and damages to its function and structural integrity (De Ronde et al. 2004; Tóth et al. 2005; Essemine et al. 2012; Zhang et al. 2018c). Therefore, the decrease of CO₂ concentration could also enhance the stability of thylakoid membrane in mulberry seedling leaves under drought stress. The stability of thylakoid membrane is affected by the stability of the PSI function, but the relatively stable structure of thylakoid membrane also improves the stability of the PSI function.

Under stress, the absorption and utilization of light energy in the PSI reaction center of plant leaves could be affected (Sun et al. 2016; Zhang et al. 2018a). In this experiment, the absorption and distribution parameters of light energy did not change significantly at drought days 0 and 5 under a CO₂ concentration of 400 μmol·mol⁻¹, indicating that the PSI function had some drought resistance, which was in agreement with the changes in Fᵢ/Fₘₒ PI_ABS and PI_TOTAL. When the drought time was 10 days, the ABS/RC of mulberry seedling leaves increased significantly and ABS/CSₘ decreased slightly, which led to a significant decrease in RC/CSₘ. This indicated that when part of the reaction center is inactivated under drought stress, mulberry seedling leaves can adapt by enhancing the light energy absorption capacity of the remaining active reaction centers, which is similar to the results of many other studies (Balabanova et al. 2016; Paukov et al. 2018). In addition, there was no significant change in ETᵣ/RC under drought stress, which further confirmed that the function of the remaining active reaction centers was enhanced. On the 10th day of drought stress, the DIᵣ/RC and DIᵣ/CSₘ of mulberry seedling leaves increased significantly, but the ETᵣ/CSₘ decreased significantly. On the 10th day of drought stress, NPQ in leaves of mulberry
seedlings increased significantly. This indicated that under drought stress, the excess excitation energy in the PSII reaction center could be reduced by increasing heat dissipation, which also could cause a decrease in the proportion of photosynthetic electron transfer energy and reduce the activity of PSII reaction centers. Compared with the treatment at a CO2 concentration of 400 μmol·mol−1, the changing trends in light absorption and distribution parameters in PSII reaction centers at 800 μmol·mol−1 was similar, but the change amplitude was greatly decreased. In particular, RC/CSm did not decrease significantly, the NPQ of Mulberry Seedling Leaves under CO2 concentration of 800 μmol·mol−1 was also significantly lower than that under 400 μmol·mol−1, indicating that the increase in CO2 concentration could affect the absorption and distribution parameters of light energy in PSII reaction centers and reduce the proportion of the leaf inactivation reaction center. Meanwhile, the proportion of energy absorbed by the PSII reaction center to the electron transfer also increased to ensure a normal energy supply and the formation of assimilation (ATP and NADPH), which provided energy for CO2 fixation.

Conclusion

Increased CO2 concentrations may reduce the stomatal conductance of mulberry seedling leaves but also improves the net photosynthetic rate and WUE. Under non-drought conditions, the increase in CO2 concentration had little effect on the photochemical activity and electron transfer of PSII in mulberry seedling leaves. Under mild drought stress (5 days of drought), the increase in CO2 had little effect on PSII function. Under severe drought stress (10 days of drought), the increased CO2 not only helped the seedlings adapt to drought stress by increasing the WUE, but also alleviated the inhibition of drought stress on the photochemical activity of PSII. This promoted the transmission of photosynthetic electrons on the PSII acceptor side, alleviated the damage from OEC on the PSII donor side, optimized the energy distribution in the PSII reaction center, and increased the number of active reaction centers and the stability of the thylakoid membrane.

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