Water use efficiency and photosynthesis of *Calamagrostis angustifolia* leaves under drought stress through CO2 concentration increase

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1. Introduction

Since the western industrial revolution in the middle and late eighteenth century, the consumption of fossil fuels, deforestation and the change of land use patterns have led to an average annual increase of approximately 2 \(\text{mol} \cdot \text{mol}^{-1}\) in atmospheric CO2 concentration (IPCC 2014). At present, the atmospheric CO2 concentration has exceeded 400 \(\text{mol} \cdot \text{mol}^{-1}\) and is expected to continue to increase to 700 \(\text{mol} \cdot \text{mol}^{-1}\) by the end of the twenty-first century (Wallace 2000; Lin et al. 2016). Simultaneously, due to the greenhouse effect caused by elevated atmospheric CO2 concentrations, the global average temperature will increase by 1.4°C ~ 5.8°C by the end of this century. In addition, climate warming will further affect the global precipitation distribution pattern, resulting in uneven distribution of seasonal precipitation and frequent occurrence of regional extreme drought events (Curtis and Wang, 1998; Nayyar and Gupta 2006). Previous climate model prediction results demonstrated that the increase of atmospheric CO2 concentration will increase the drought degree and the frequency of extreme drought events at middle and high latitudes (Gifford 1995; Ziska and Bunce 1998; Zhou et al. 2001; Wu et al. 2004; Ainsworth and Long 2005), while the frequency and scope of high temperature ‘heat wave’ events increase daily (Varga et al. 2017; Ma et al. 2018; Ye et al. 2018; Zhang et al. 2019). Drought and increased CO2 concentration will jointly affect the physiological and ecological processes of plants (Xu et al. 2016; Xu et al. 2017). Drought stress is one of the important limiting factors in agricultural production (Pukacki and Kamińska-Rożek et al. 2005), which inadvertently leads to the decline of crop yield and quality. Plant photosynthesis is one of the more sensitive processes to drought. Drought stress often leads to leaf wilting, leaf curling and chlorophyll content reduction. Drought stress also leads to the decrease of PSI and PSII activities, the obstruction of photosynthetic electron transfer and the accumulation of excess energy (Nikolaeva et al. 2010; Albert et al. 2011; Zhang et al. 2018). In addition, drought stress could also lead to the decrease of PSII and PSI activities, the obstruction of photosynthetic electron transfer and the accumulation of excess energy (Schwanz and Polle 2001; Gill and Tuteja 2010). The excess electrons and energy could deteriorate the increase of reactive oxygen species (ROS) content in cells, as well as the excess ROS breaks the redox balance in plants, causing membrane peroxidation damage (Shanmugam et al. 2013; Wang et al. 2017). Therefore, it would be beneficial to improve the simulation accuracy of the ecological process model, as well as accurately predict the far-reaching impact of future climate change on the structure and function of different types of ecosystems, through collaborative response mechanism in-depth exploration of plants to multiple environmental factors, such as doubled CO2 concentrations, high temperature and water. In particular, the research on the response of farmland ecosystem to climate change,
which is directly related to human food security, has concerned scholars widely (Fleisher et al. 2008; Sun et al. 2012).

Many studies have demonstrated that the increase of CO₂ concentration could alleviate the inhibition of abiotic stress on plant growth and physiological functions (Morison and Gifford 1983; Medina et al. 2016; Li et al. 2017; Wei et al. 2018). The increase of intercellular CO₂ concentration (Cᵢ) caused by the increase of CO₂ concentration could compensate for the CO₂ limitation (Pazzagli et al. 2016), caused by the decrease of stomatal conductance under stress, while the increase of CO₂ concentration could reduce transpiration through stomatal conductance (Gₛ) reduction, to improve the water use efficiency of plant leaves under stress (Fleisher et al. 2008; Dong et al. 2015; Varga et al. 2017). The increase of CO₂ concentration relatively increased the carbohydrate concentration under drought stress, resulting in an increase of cell osmotic potential, which improved the drought resistance of plants (Katsube et al. 2006). C. angustifolia is a gramineous herb, which mainly exists in the plain low wetlands of Northeast China, North China, Inner Mongolia as well as other similar regions. It is a typical meadow constructive plant and dominant plant in Sanjiang Plain (Zhang et al., 2018; Wei et al. 2019). The leaves of C. angustifolia could not only be used as feed, but it also has antioxidant activity, which has important nutritional value. However, no report exists regarding the effect of elevated CO₂ concentration on photosynthetic function of C. angustifolia seedlings under drought stress. At present, the research on effects of elevated CO₂ concentration on photosynthesis of plants under drought stress is mainly focused on stomata and photosynthetic gas exchange, while the research on the effect of elevated CO₂ concentration on PSII function in plant light response under drought stress is significantly lower in amount. Therefore, the effects of elevated CO₂ concentration on photosynthetic gas exchange, instantaneous water use efficiency and PSII function of C. angustifolia seedling leaves under drought stress were studied through natural drought method utilization, in order to provide a certain amount of basic data for the mechanism discovery of elevated CO₂ concentrations on plant photosynthesis under drought stress.

2. Materials and methods

2.1. Test materials and treatment

The experiment was conducted at the Water Conservancy Institute of nature and ecology in Heilongjiang Academy of Sciences in 2020. The variety of C. angustifolia was a local meadow variety from Sanjiang Plain of Heilongjiang Province. The seeds were one-year C. angustifolia 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 (two seedlings were planted) of C. angustifolia seedlings with relatively uniform growth were selected as experimental materials, five pots were placed in climatic chamber (Conviron E8, Canada) with CO₂ concentrations of 370 μmol·mol⁻¹ and five pots were placed in another climatic chamber with CO₂ concentrations of 700 μmol·mol⁻¹. The climatic chamber 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 C. angustifolia 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.

2.2. Determination parameters and methods

5 and 10 days after treatment, the growth characteristics of plants were measured, including plant height of main stem, root length, leaf length and leaf width of the last fully expanded leaf, while the leaf area (leaf length × leaf width × 0.75) was calculated. The root of the plant was washed clean and divided into the ground part and the underground part. The water on the plant surface was absorbed with filter paper, while the fresh weight was weighed with an analytical balance. Consequently, the plant was placed into the oven at 105°C for 30 min and dried to constant weight at 80°C to weigh the dry weight. Root shoot ratio = root dry weight / seedling dry weight.

The determination of physiological characters was as follows: Three plants were selected for each weight and the last fully developed leaf was removed for determination. Chlorophyll content was determined with SPAD-502 device, manufactured by Minolta company. The activity of peroxidase (POD) was determined through guaiacol colorimetry. The content of Pro was determined through acid ninhydrin colorimetry (Li 2000).

2.2.1. Determination of photosynthetic gas exchange parameters

The reciprocal second pieces of completely unfurled mulberry seedlings on different drought days were selected. The net photosynthetic rate (Pₙ), stomatal conductance (Gₛ), transpiration rate (Tᵣ), and intercellular CO₂ concentration (Cᵢ) 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 = Pₙ/Tᵣ. The test was repeated five times.

2.2.2. Determination of chlorophyll fluorescence kinetics (OJIP) curve

The penultimate fully expanded leaf of C. angustifolia seedlings with different treatments was selected, while the dark adaptation clip was used for 30 min. Subsequently to dark adaptation, conducted with Hansatech device, which was the multi-functional plant efficiency analyzer (Handy-PEA), the OJIP curve of the leaves after dark adaptation was produced. Five repetitions of each treatment were measured, while the average value of these five repetitions was utilized.
to produce the OJIP curve. The O, J, I and P points were produced by the relative fluorescence intensity as expressed as $F_O$, $F_J$, $F_I$ and $F_P$, respectively. The OJIP curves were normalized according to the $V_{O,P}=\left(F_{O}-F_{P}\right)/\left(F_{O}-F_{P}\right)$, $V_{O,J}=\left(F_{O}-F_{J}\right)/\left(F_{O}-F_{P}\right)$ and $V_{O,K}=\left(F_{O}-F_{K}\right)/\left(F_{O}-F_{P}\right)$, respectively, while the $V_{O,P}$, $V_{O,J}$ and $V_{O,K}$ curves were obtained, signifying the relative variable fluorescence $V_{i}$ of point $i$ (0.15 ms) on $V_{O,P}$ curve and the relative variable fluorescence $V_{K}$ of point K (0.3 ms) on $V_{O,J}$ curve and the relative variable fluorescence $V_{L}$ of point L (0.15 ms) on $V_{O,K}$ curve. Where $F_{i}$ is the relative fluorescence intensity at each time point on the OJIP curve. The $V_{O,P}$, $V_{O,J}$ and $V_{O,K}$ curves of *Calamagrostis angustifolia* seedlings under different drought days were compared to CK and 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 ($F_{i}/F_{m}$), performance index of PSII based on absorption (PI$_{ABS}$), performance index of electron flux to the final PSI electron acceptors, such as for PSI and PSI (PI$_{total}$), absorption flux per reaction center (ABS$\cdot$RC), electron transport flux per reaction center (ET$\cdot$RC), dissipated energy flux per reaction center (ET$\cdot$RC), dissipated energy flux per excited cross section (ET$\cdot$RC$\cdot$C$_{m}$), electron transport flux per excited cross section (ET$\cdot$RC$\cdot$C$_{s}$) and the number of active response centers per unit area (RC$\cdot$C$_{m}$). The JIP-test analysis of OJIP curves was based on the method of Strasser et al. (1995).

### 2.3. Data analysis

Microsoft Excel (Version 2020, Microsoft Excel, USA) and SPSS (Version 22, USA) were used to conduct statistical analyses on the data. The data in the figures are denoted as the mean ± standard deviation (SD). A two-way analysis of variance (ANOVA) was used to detect the significant differences of trace element concentration, AMF inoculation and their interaction on all variables.

### 3. Results and analysis

#### 3.1. Growth characteristics

Under drought for 5 d, the plant height, leaf length, leaf width and total biomass of under high CO$\text{2}$ concentration (700 μmol·mol$^{-1}$) were compared with those under normal CO$\text{2}$ concentration (370 μmol·mol$^{-1}$). The relative traits of growth characteristics increased by 10.7%, 4.3% and 8.9%, respectively. There were no significant differences in root length and root-shoot ratio. Under drought for 10 d, the plant height, leaf length, leaf width and total biomass of leaves under high CO$\text{2}$ concentration (700 μmol·mol$^{-1}$) were compared with those under normal CO$\text{2}$ concentration (370 μmol·mol$^{-1}$). The relative traits of growth characteristics increased by 17.9%, 5.3% and 12.7%, respectively. (Figure 1).

Table 1 shows that the effect of high CO$\text{2}$ concentration on root/shoot ratio of *C. angustifolia* was insignificant, however the effect on growth parameters was significant ($P<0.01$). Drought stress had a significant effect on the growth parameters of *C. angustifolia* ($P<0.01$), but it showed no significant interaction with CO$\text{2}$ concentration.

#### 3.2. Physiological characters

As the drought time increased, under drought for 5 d the content of SPAD decreased by 30.7%, the content of MDA increased by 48.0%, the content of proline increased by 1031.6% and the activity of pod increased by 36.5% under normal CO$\text{2}$ concentration (370 μmol·mol$^{-1}$). However, throughout the entire treatment, MDA and pod in leaves of *C. angustifolia* seedlings treated with 700 μmol·mol$^{-1}$ CO$\text{2}$ were significantly higher than the seedlings treated with 370 μmol·mol$^{-1}$ of CO$\text{2}$. Specifically, as drought time increased, the increase of MDA in leaves of *C. angustifolia* seedlings treated with 700 μmol·mol$^{-1}$ CO$\text{2}$ was significantly higher than the seedlings treated with 370 μmol·mol$^{-1}$ CO$\text{2}$ (Figure 2).

Under the drought stress, MDA, POD, and Proline in the leaves of *C. angustifolia* increased significantly, and the content of Spad showed a sharp decrease. Drought stress had a significant effect on the MDA, POD, Proline and Spad of *C. angustifolia* ($P<0.01$), but it showed no significant interaction with CO$\text{2}$ concentration. (Table 2).

#### 3.3. Photosynthetic gas exchange parameters

At 0 d after drought, although $G_{s}$ and $T_{r}$ in leaves of *C. angustifolia* seedlings treated with 700 μmol·mol$^{-1}$ CO$\text{2}$ were slightly lower than seedlings treated with 370 μmol·mol$^{-1}$ CO$\text{2}$, $P_{n}$ increased by 21.30% ($P<0.01$), while WUE also increased significantly under drought stress. As drought time increased, $P_{n}$, $G_{s}$, and $T_{r}$ in leaves of *C. angustifolia* seedlings decreased significantly. However, no significant difference in $G_{s}$ and $T_{r}$ occurred in leaves of *C. angustifolia* seedlings under different CO$\text{2}$ concentrations, except that $T_{r}$ in leaves of *C. angustifolia* seedlings treated with 700 μmol·mol$^{-1}$ CO$\text{2}$ was significantly higher than at 370 μmol·mol$^{-1}$ CO$\text{2}$ concentration at 5d of drought. However, $P_{n}$ and WUE of *C. angustifolia* seedlings treated with 700 μmol·mol$^{-1}$ CO$\text{2}$ were significantly higher than the seedlings treated with 370 μmol·mol$^{-1}$ CO$\text{2}$. Specifically, as drought time increased, the increase of WUE of *C. angustifolia* seedlings treated with 700 μmol·mol$^{-1}$ CO$\text{2}$ was significantly higher than the seedlings treated with 370 μmol·mol$^{-1}$ CO$\text{2}$.

The effect of CO$\text{2}$ concentration on net photosynthetic rate, stomatal conductance, transpiration rate and intercellular CO$\text{2}$ concentration of *C. angustifolia* was significant ($P<0.01$, Table 3). Drought stress also had a significant effect on these parameters ($P<0.01$). However, these two factors had no significant effect on intercellular CO$\text{2}$ concentration of *C. angustifolia* ($P>0.05$), and there was no significant interaction effect between these two factors on the photosynthetic gas exchange parameters. (Figure 3).

#### 3.4. OJIP curve and relative fluorescence intensity of characteristic points (O and P)

Compared to the 0 d of drought, the OJIP curves of *C. angustifolia* seedlings at 370 and 700 μmol·mol$^{-1}$ CO$\text{2}$ concentrations changed less at 5d of drought. At 10 d of drought, the relative fluorescence intensity $F_{o}$ at o-point on the OJIP curve of *C. angustifolia* seedlings at 370 μmol·mol$^{-1}$ CO$\text{2}$ concentration increased, while the relative fluorescence intensity $F_{p}$ at P-point decreased...
significantly (Figure 4A). However, at 10 d of drought, the change range of OJIP curve of C. angustifolia seedlings at 700 μmol·mol⁻¹ CO₂ concentration was

significantly lower than at 370 μmol·mol⁻¹ CO₂ concentration (Figure 4B).

Table 1. Two-way ANOVAs examining the effects of high CO₂ concentration, Drought stress and their interaction (CO₂×Drought stress) on plant height, root length, total biomass, Leaf length, Leaf width and root/shoot ratio.

| CO₂×Drought stress | F     | P    | F     | P    |
|---------------------|-------|------|-------|------|
| Plant height        | 19.15 | <0.001 | 12.71 | <0.001 | 0.55 | 0.65 |
| Root length         | 49.11 | <0.001 | 128.38 | <0.001 | 1.73 | 0.20 |
| Total biomass       | 61.61 | <0.001 | 12.46 | <0.001 | 1.78 | 0.19 |
| Leaf length         | 62.91 | <0.001 | 12.62 | <0.001 | 1.05 | 0.40 |
| Leaf width          | 61.71 | <0.001 | 19.56 | <0.001 | 2.83 | 0.07 |
| Root/shoot ratio    | 8.83  | 0.02  | 45.04 | <0.001 | 1.59 | 0.23 |

Drought stress showed significant effect on Fo and Fm (P < 0.001). However, there was no significant interaction effect between these two factors on Fo and Fm.
3.5. PSII photochemical efficiency

$F_v/F_m$, $P_{I_{ABS}}$ and $P_{I_{TOTAL}}$ of C. angustifolia seedlings at 370 and 700 μmol-mol⁻¹ CO₂ concentrations had insignificant difference at 0 d of drought. $F_v/F_m$, $P_{I_{ABS}}$ and $P_{I_{TOTAL}}$ of C. angustifolia seedlings decreased as the drought days increases, but changed slightly at 5d of drought, while no significant difference existed between 370 and 700 μmol·mol⁻¹ CO₂ concentrations. At 10 d of drought, $F_v/F_m$, $P_{I_{ABS}}$ and $P_{I_{TOTAL}}$ at 700 μmol·mol⁻¹ concentration were 14.65% (P<0.05), 19.22% (P<0.05) and 42.54% (P<0.05) higher than at 370 μmol·mol⁻¹ concentration, respectively.

High CO₂ concentration and drought stress had significant effect on $F_v/F_m$, $P_{I_{ABS}}$ and $P_{I_{TOTAL}}$ of C. angustifolia (P<0.01), which is shown in Table 5, but they had no significant interaction effect on these parameters.

3.6. Standardized O-P curve and relative variable fluorescence $V_j$ of J point (2 ms)

The relative fluorescence intensity of points O and P on the OJIP curve of C. angustifolia seedlings with different treatments were defined as 0 and 1, respectively. After the OJIP curve was standardized, it could be observed that the relative variable fluorescence ($V_j$) of J point on the standardized O-P curve of C. angustifolia seedlings at 5d and 10 d increased to various degrees compared to the 0 d of drought, while the $V_j$ at 10 d of drought increased to various degrees. The increase range of $V_j$ at 5d was higher than at 5d.

Quantitative analysis of $V_j$ showed that insignificant difference existed between 370 and 700 μmol·mol⁻¹ CO₂ concentrations. At 10 d of drought, $V_j$ in leaves of C. angustifolia seedlings increased significantly compared to 0 d of drought, but the increase of $V_j$ in leaves of C. angustifolia seedlings under 700 μmol·mol⁻¹ CO₂ concentration was lower than the increase under 370 μmol·mol⁻¹ CO₂ concentration. (Figure 6A).
It is shown in Table 6 that the effect of CO₂ concentration and drought stress on $V_J$, $V_K$ and $V_L$ of *C. angustifolia* was significant ($P<0.01$), but their interaction effect on $V_J$ and $V_K$ was not significant.

3.7. Standardized O-J curve and relative variable fluorescence $V_K$ at k point (0.3 ms)

In order to further analyze the change of relative variable fluorescence of K point at 0.3 ms, the relative fluorescence intensity of O point and J point on the OJIP curve of different treatments were defined as 0 and 1, respectively. After the O-J curve was standardized (Figure 6B), the relative variable fluorescence $V_K$ of K point at 0.3 ms on the standardized O-J curve of different treatments presented apparent differences. Under drought stress, the increase of in leaves of *C. angustifolia* seedlings was significantly higher at 10 d than at 5d, but the increase of $V_K$ in leaves of *C. angustifolia* seedlings under 370 μmol·mol$^{-1}$ CO₂ concentration was significantly higher than at under 700 μmol·mol$^{-1}$ CO₂ concentration. (Table 7).

The results of quantitative analysis of $V_K$ demonstrated that no significant difference exited between 370 μmol·mol$^{-1}$ CO₂ and 700 μmol·mol$^{-1}$ CO₂ at 0 d and 5d of drought, but at 10 d of drought, $V_K$ of 700 μmol·mol$^{-1}$ CO₂ was lower than of 370 μmol·mol$^{-1}$ CO₂ by 6.42% ($P<0.05$).

3.8. Standardized O-K curve and l point (0.15 ms) relative variable fluorescence $V_L$

In order to further analyze the change of relative variable fluorescence $V_L$ of L point at 0.15 ms, the relative fluorescence intensity of O point and K point on the OJIP curve of leaves of *C. angustifolia* seedlings seedlings...
under different treatments were defined as 0 and 1, respectively. After the O-K curve was standardized, it could be observed (Figure 6C), as the dry days increased, 370 $V_L$ in leaves of C. angustifolia seedlings increased significantly at the concentration of 100 $\mu$mol·mol$^{-1}$ CO$_2$, but changed slightly at the concentration of 700 $\mu$mol·mol$^{-1}$ CO$_2$.

The results of quantitative analysis of $V_L$ (Figure 6C) demonstrated that under 370 $\mu$mol·mol$^{-1}$ CO$_2$ concentration, the $V_L$ of C. angustifolia seedlings leaves increased by 2.74% and 9.26% ($P<0.05$), respectively, at 5d and 10 d after drought, compared to the concentration at 0 d after drought, but no significant change occurred in $V_L$ of C. angustifolia seedlings leaves under 700 $\mu$mol·mol$^{-1}$ CO$_2$ concentration, compared to concentration at 0 d after drought at 5d and 10 d.

3.9 Parameters of energy distribution per unit reaction center and unit area

At 370 $\mu$mol·mol$^{-1}$ CO$_2$ concentration, the energy allocation parameters per unit reaction center and per unit cross-sectional area did not change significantly at 5d compared to 0 d, but at 10 d ABS/RC, DI$_o$/RC and DI$_o$/CS$_m$ increased by 32.98% ($P<0.01$), 30.56% ($P<0.01$) and 28.16% ($P<0.01$), respectively. ET$_o$/RC and ABS/CS$_m$ did not change significantly, but ET$_o$/CS$_m$ decreased by 29.59% ($P<0.01$). Under 700 $\mu$mol·mol$^{-1}$ CO$_2$ concentration (Figure 7A), the change trend of each energy allocation parameter in leaves of C. angustifolia seedlings was similar to of the trend at 370 $\mu$mol·mol$^{-1}$ CO$_2$ concentration, but the change amplitude was significantly lower than of 370 $\mu$mol·mol$^{-1}$ CO$_2$ concentration. Also, no significant difference occurred in each energy allocation parameter between 0, 5, and 10 d of drought.

High CO$_2$ concentration had no significant effect on ABS/CS$_m$ ($P>0.05$), but had significant effect on ABS/RC, ET$_o$/RC, DI$_o$/RC, ET$_o$/CS$_m$ and DI$_o$/CS$_m$ ($P<0.001$). Drought stress had significant effect on ABS/RC, ET$_o$/RC, DI$_o$/RC, ABS/CS$_m$, ET$_o$/CS$_m$ and DI$_o$/CS$_m$ ($P<0.001$), but they had no significant interaction effect on these parameters.

This could be observed from the change of RC/CS$_m$ in the number of active reaction centers per unit area (Figure 7B). At 5d of drought, the RC/CS$_m$ in the leaves of C. angustifolia seedlings at 370 and 700 $\mu$mol·mol$^{-1}$ CO$_2$ concentrations did not change significantly compared to 0 d of drought, but at 10 d of drought, the RC/CS$_m$ in the leaves of C. angustifolia seedlings at 370 $\mu$mol·mol$^{-1}$ CO$_2$ concentrations decreased.
Figure 5. Effects of high CO₂ concentration, drought and interaction on $F_{v}/F_{m}$, $P_{\text{ABS}}$ and $P_{\text{total}}$ of *C. angustifolia* seedlings 370 and 700 in the figure indicated that CO₂ concentrations were 370 and 700 μmol·mol$^{-1}$, respectively.

Note: Data in the figure are the mean ± SE; values followed by different lowercase letters indicate a significant difference ($p<0.05$).
Table 4. Two-way ANOVAs examining the effects of high CO2 concentration, Drought stress and their interaction (CO2×Drought stress) on Fr and Fm.

| CO2               | Drought stress | CO2×Drought stress |
|-------------------|----------------|--------------------|
| F     P            | F     P        | F     P           |
| Fr    15.77 <0.001 | 81.32 <0.001  | 2.49 0.09         |
| Fm    0.44 0.43    | 22.50 <0.001  | 0.11 0.93         |

Table 5. Two-way ANOVAs examining the effects of high CO2 concentration, Drought stress and their interaction (CO2×Drought stress) on Fr/Fm and PiABS.

| CO2 | Drought stress | CO2×Drought stress |
|-----|----------------|--------------------|
|     | F     P        | F     P           |
| Fr/Fm | 14.83 <0.001 | 144.36 <0.001     | 3.42 0.04         |
| PiABS | 35.52 <0.001 | 102.44 <0.001     | 0.06 0.98         |
| PiTOTAL | 24.33 <0.001 | 110.21 <0.001   | 1.25 0.33         |

by 9.09% (P<0.01), compared with 0 d of drought, while at 700 μmol·mol−1 CO2 concentrations, the RC/CSm in the leaves of C. angustifolia seedlings decreased. However, no significant decrease occurred at the concentration of 700 μmol·mol−1 CO2.

4. Discussion

4.1. Compensation effect of high CO2 concentration on growth and development of C. angustifolia seedlings under drought

The atmospheric CO2 concentration and drought increase have become the trend of climate change in the future. A high number of studies have demonstrated that drought has a significant inhibitory effect on plant growth and development. Li et al. (2020) found that as the water content continuously decreased, the growth of plant height, total biomass, root length, root shoot ratio, above ground and underground fresh weight of Coreopsis tinctoria followed a downward trend. Han et al. (2019) demonstrated that under normal irrigation, Punicum milacium seedlings grew best, with the highest plant height, stem diameter, stem node number, leaf number, maximum leaf area, fresh weight and dry weight of stem, leaf and ear. Under drought stress, all morphological indexes and biomasses of seedlings significantly decreased. Another studies results demonstrated that drought inhibited the growth and development of wheat seedlings to a high extent, mainly in tiller numbers, seedling height, leaf area and other traits (Wang et al. 2017; Chanvan et al. 2019). In our results demonstrated that the growth rate, seedling height, leaf length, leaf width and leaf area of C. angustifolia increased significantly under high CO2 concentration, while the increase of photosynthetic area was conducive to the accumulation of dry matter, consequently increasing the total biomass parts of C. angustifolia seedlings. It could be observed that high CO2 concentration could partly make up for the effect of drought on the growth and development of wheat seedlings.

Compared to 370 μmol·mol−1 CO2, the root length of C. angustifolia seedlings increased significantly. It could be observed that the effect of high CO2 concentration on wheat root growth was significantly apparent under drought conditions, which was consistent with the research results of Li et al. (2003). Furthermore, high CO2 concentration had a higher promoting effect on root growth.

4.2. Compensation of high CO2 concentration for growth and development of C. angustifolia under drought

The physiological mechanism of action was that cell membrane was the first damaged site of plant under drought and other stresses (Zhao et al. 2008; Zhao et al. 2015). Intracellular peroxidase (POD) was closely related to plant stress resistance, which could reduce the accumulation of reactive oxygen species in plants under stress (Zhu et al. 2009). Malondialdehyde (MDA) is one of the most important products of membrane lipid peroxidation, while the corresponding content could directly reflect the degree of cell membrane damage (Bowes 1993; Ainsworth and Long 2005; Zhang and Dang 2005; Ke 2007; Pazzagli et al. 2016). Proline is a type of osmoregulation substance in plants. The increase of proline content could change the permeability of cell plasma membrane, reduce the osmotic potential of cells, reduce the water loss of cells, as well as enhance the stress resistance of plants (Jin et al. 2019). The results demonstrated that as the drought time increased, compared to 370 μmol·mol−1 treatment, MDA content increased and POD activity decreased under 700 μmol·mol−1 treatment. It could be observed that high CO2 concentration had a certain protective effect on the oxidative damage of cells, consequently reducing the damage of cell membrane caused by drought (Rao et al. 1995; Ren and Chen 2001; Susan et al. 2016).

Certain researchers considered that the increase of CO2 concentration would lead to the decrease of Gs and the partial closure of stomata (Murray 1995; Alejandro et al. 2005; Ainsworth and Rogers 2007; Prasad et al. 2009). Curtis and Wang (1998) also demonstrated that the doubling of CO2 concentration could reduce the stomatal conductance by approximately 11% (Curtis and Wang 1998), while the increase of CO2 concentration would also reduce the stomatal density of winter wheat (Woodward and Kelly 1995). In this experiment, Gs and Tr in leaves of C. angustifolia seedlings at 700 μmol·mol−1 CO2 concentration were lower than at 370 μmol·mol−1-CO2 concentration at 0 d of drought, and the Gs at 370 μmol·mol−1 CO2 concentration were lower than at 700 μmol·mol−1 CO2 concentration under drought stress, but Gs increased significantly, which indicated that Gs was not the limiting factor of Pn increase under doubled CO2 concentration, whereas the increase of CO2 concentration could compensate the decrease of CO2 caused by the decrease of Gs, consequently promoting photosynthesis. In addition, elevated CO2 concentration could also increase Rubisco activity and carboxylation direction (Li et al. 2011), or improve photosynthetic capacity by increasing substrate and inhibiting photorespiration (Li et al. 2011), which would be similar to the results of Peng et al. (2004).

Drought stress could lead to the increase of ABA content in plants (Davies and Zhang 1991; Peng et al. 2004; Peng et al. 2004). ABA adapts to drought stress by inducing stomatal closure and increasing water use efficiency (Moretti et al. 2010). CO2 is not only the substrate of photosynthesis, but it is also the regulator of stomata. The decrease of Gs caused by the increase of CO2 concentration had quite lower effect on CO2 absorption than on water vapor diffusion. Therefore, although the increase of CO2 concentration reduced Gs, it alleviated drought stress by reducing transpiration loss and improving water use efficiency (Morison and Gifford 1983; Wu and Wang 2000; Liu et al. 2005; Ainsworth and Rogers
Figure 6. Effects of high CO$_2$ concentration, drought and interaction on $V_J$, $V_L$ and $V_K$ of $C. angustifolia$ seedlings 370 and 700 in the figure indicated that CO$_2$ concentrations were 370 and 700 μmol·mol$^{-1}$, respectively.

Note: Data in the figure are the mean ± SE; values followed by different lowercase letters indicate a significant difference ($p<0.05$).
between elevated CO2 concentration and drought stress on PF of NADPH) feedback could inhibit the light reaction process under drought stress by increasing the water use efficiency of leaves of C. angustifolia while reducing the non-photochemical quenching coefficient, to reduce the damage degree of drought stress to a certain extent.

Under stress conditions, the blocking sites of photosynthetic electron transfer often occurred in the electron donor and receptor sides of PSII reaction center (Jiang et al. 2008; Shuang et al. 2009). In certain studies, it was discovered that the main reason for elevated CO2 concentration that alleviated the photosynthetic limitation of plant leaves was relation to the promotion of photosynthetic electron transfer (Zhu et al. 2018). In order to analyze the effect of elevated CO2 concentration on photosynthetic electron transfer ability of C. angustifolia seedlings leaves under drought stress, the OJIP curve was standardized with O-P and O-J. The increase of J-point relative to variable fluorescence V1 at 2 ms indicated that the electron transfer from QA to QB in photosynthetic electron transport chain was blocked (Zhang et al. 2017; Xu et al. 2018). The increase of relative variable fluorescence V6 at 0.3 ms on the standardized O-J curve was considered to be a specific marker of the damage of OEC activity on the electron donor side of PSII (Zhang et al. 2012; Zhang et al. 2016). In the experiments, V1 and V6 in leaves of C. angustifolia seedlings increased in various degrees under drought stress, indicating that the decrease of PSII photochemical activity in leaves of C. angustifolia seedlings under drought stress was related to the blocked electron transfer on the donor and receptor sides of PSII (Cui et al. 2017). Although no significant difference occurred in V1 and V6 at 370 and 700 μmol·mol−1 CO2 concentration at 0 d and 5d of drought, V1 and V6 at 700 μmol·mol−1 CO2 concentration at 10 d of drought were significantly lower than at 370 μmol·mol−1 CO2 concentration treatment. This demonstrated that elevated CO2 concentration could alleviate the damage of OEC in leaves of C. angustifolia seedlings under severe drought stress, while promoting the electron transfer from QA to QB in PSII receptor side of leaves of C. angustifolia seedlings under drought stress. This was consistent with the results of Cui study (Cui et al. 2017), in which, elevated CO2 concentration increased the abundances of PsbJ, PSII chlorophyll a-b binding protein and PSI reaction center protein sub-units in Cucumber seedling leaves under drought stress, which contributed to improve the electron transfer rate of Cucumber seedling leaves under drought stress. In addition, VI in leaves of C. angustifolia seedlings at 700 μmol·mol−1 CO2 concentration was also significantly lower than at 370 μmol·mol−1 CO2 concentration at 10 d of drought. Under the treatment of 700 μmol·mol−1 CO2 concentration, the increase of VI was considered to be an important indicator of the change of thylakoid membrane fluidity and the destruction of the corresponding function and structural integrity (De Ronde et al. 2004; Essemine et al. 2012; Cui et al. 2017). Therefore, the increase of CO2 concentration could also improve the stability of thylakoid membrane in the leaves of C. angustifolia seedlings under drought stress. The stability of thylakoid membrane was not only affected by the fundamental stability of PSII, but it was also relatively stable. The structure could also improve the functional stability of PSII.

Stress could affect the absorption and utilization of light energy in PSII reaction center of plant leaves (Tóth et al. 2005; Sun et al. 2016). In the experiments, at 370 μmol·mol−1 CO2 concentration, the light energy absorption and distribution parameters did not change significantly at 5d compared to 0 d, indicating that PSII function had a certain

### Table 6. Two-way ANOVAs examining the effects of high CO2 concentration, drought stress and their interaction (CO2×Drought stress) on V1 and V6.

| CO2 | Drought stress | CO2×Drought stress |
|-----|----------------|---------------------|
|     | F              | P                   | F | P |
| V1  | 82.15 <0.001   | 80.76 <0.001        | 3.21 | 0.04 |
| V6  | 24.31 <0.001   | 15.55 <0.001        | 0.05 | 0.95 |
| V1  | 15.36 <0.001   | 6.23 <0.001         | 1.58 | 0.23 |

### Table 7. Two-way ANOVAs examining the effects of high CO2 concentration, drought stress and their interaction (CO2×Drought stress) on ABS/RC, ET/RC, DI/RC, ABS/CSm, ET/CSm, and DI/CSm.

|                     | CO2 | Drought stress | CO2×Drought stress |
|--------------------|-----|----------------|---------------------|
|                    | F   | P              | F | P |
| ABS/RC            | 23.12 | <0.001      | 42.25 | <0.001 |
| ET/RC             | 99.35 | <0.001      | 82.60 | <0.001 |
| DI/RC             | 23.41 | <0.001      | 108.11 | <0.001 |
| ABS/CSm           | 0.85  | 0.32         | 38.37 | <0.001 |
| ET/CSm            | 42.55 | <0.001      | 106.04 | <0.001 |
| DI/CSm            | 27.97 | <0.001      | 156.18 | <0.001 |

2007; Sun et al. 2014; Pazzagli et al. 2016). Hamerlynck et al. (2000) demonstrated that a significant interaction existed between elevated CO2 concentration and drought stress on Pn and Gs, while high CO2 concentration could improve the adaptability of plants to drought (Hamerlynck et al. 2000). Similar results were obtained in the conducted experiments. Under drought stress, Gs in leaves of C. angustifolia seedlings decreased, along with Pn and Tr, but Tr decreased faster than Pn, which led to the increase of WUE in leaves of C. angustifolia seedlings. Under different drought days, WUE in leaves of C. angustifolia seedlings under 700 μmol·mol−1 CO2 concentration was significantly higher than at 370 μmol·mol−1 CO2 concentration. The treatment of 700 μmol·mol−1 CO2 concentration indicated that the increase of CO2 concentration could improve the adaptability to drought stress by increasing the water use efficiency of leaves of C. angustifolia seedlings.

The accumulation of assimilative capacity (ATP and NADPH) feedback could inhibit the light reaction process (Liu et al. 2006; Zhang et al. 2012; Jiang et al. 2016; Chen et al. 2017), resulting in the decrease of PSII reaction center activity. In the experiments, Fv/Fm, PIABS and PIFtotal decreased as the drought time increased, especially PIABS and PIFtotal which indicated that PSII photochemical activity decreased under drought stress. Scientists have found that elevated CO2 concentration could affect PSII structure, photosynthetic energy conversion and electron transfer of plant leaves (Xu et al. 2016). However, the Fv/Fm, PIABS and PIFtotal of C. angustifolia seedlings were not significantly affected by elevated CO2 concentration at 0 d and 5d of drought, but at 10 d of drought, Fv/Fm, PIABS and PIFtotal of C. angustifolia seedlings at 700 μmol·mol−1 CO2 concentration were significantly higher than at 370 μmol·mol−1 CO2 concentration. This indicated that the increase of CO2 concentration could alleviate the photoinhibition of PSII in leaves of C. angustifolia seedlings under severe drought stress. This was similar to the results of Li et al. (2008). It was found that elevated CO2 concentration could improve the maximum quantum yield and photochemical quenching coefficient of PSII in Cucumber Seedling Leaves under drought stress, while reducing the non-photochemical quenching coefficient, to reduce the damage degree of drought stress to a certain extent.
drought resistance ability, which was consistent with the changes of $F_v/F_m$, $PI_{ABS}$, and $PI_{total}$. However, at 10 d of drought, the ratio of $ABS/CS_m$ increased significantly, while the ratio of $RC/CS_m$ decreased slightly, which led to the decrease of $RC/CS_m$ at 10 d of drought. The results demonstrated that the leaves of *C. angustifolia* seedlings could adapt to drought stress by enhancing the light energy absorption capacity of the remaining active reaction centers, when the partial reaction centers of leaves would be inactivated under drought stress, which was similar to most research results (Balabanova et al. 2016; Paunov et al. 2018). In addition, the $ETo/RC$ ratio did not change significantly under drought stress, which further indicated that the function of residual active reaction center in leaves of *C. angustifolia* seedlings was enhanced under drought stress. However, $DI_o/RC$ and $DI_o/CS_m$ in leaves of *C. angustifolia* seedlings increased significantly at 10 d of drought stress, while $ETo/CS_m$ decreased significantly, which indicated that *C. angustifolia* seedlings could reduce the excess excitation energy of PSII reaction center by increasing heat dissipation under drought stress. This also led to the decrease of the proportion of photosynthetic electron transfer energy and the decrease of PSII reaction center activity. Differently from 370 $\mu$mol·mol$^{-1}$ CO$_2$ treatment, the variation trend of PSII anti center light absorption and distribution parameters in 700 $\mu$mol·mol$^{-1}$ CO$_2$ treatment was similar to 370

**Figure 7.** A. Effects of high CO$_2$ concentration, drought and interaction on Parameters of energy distribution per unit reaction center and unit area of *C. angustifolia* seedlings 370 and 700 in the figure indicated that CO$_2$ concentrations were 370 and 700 $\mu$mol·mol$^{-1}$, respectively. B. Effects of high CO$_2$ concentration, drought and interaction on Parameters of energy distribution per unit reaction center and unit area of *C. angustifolia* seedlings 370 and 700 in the figure indicated that CO$_2$ concentrations were 370 and 700 $\mu$mol·mol$^{-1}$, respectively.

Note: *There was significant difference between treatment and drought day 0 * ($P < 0.05$), * * ($P < 0.01$), and NS ($P > 0.05$); Data in the figure are the mean ± SE; values followed by different lowercase letters indicate a significant difference ($P<0.05$).
μmol·mol⁻¹ CO₂ concentration treatment, but the change range was significantly reduced. Specifically, RC/CS_m did not decrease significantly during drought, indicating that elevated CO₂ concentration could affect the light absorption and distribution parameters of PSII reaction center in leaves of C. angustifolia seedlings under drought stress, as well as reduce the proportion of inactivated reaction center in leaves of C. angustifolia seedlings under drought stress, while relatively increasing PSII. The proportion of light energy absorbed by reaction center for electron transfer could ensure the normal energy supply and the generation of assimilation power (ATP and NADPH) in PSII electron transfer, which would also provide energy supply for CO₂ fixation.

5. Conclusions

Although the stomatal conductance of C. angustifolia seedlings decreased as the CO₂ concentration increased, the net photosynthetic rate and water use efficiency of C. angustifolia seedlings were higher compared to C. angustifolia seedlings. The effect of elevated CO₂ concentration on PSII photochemical activity and electron transfer of C. angustifolia seedlings was lower under non drought conditions. Under mild drought stress (5d), elevated CO₂ concentration had little effect on PSII function, but it had under severe drought stress (10 d). The CO₂ concentration could not only be adapted to drought stress through water use efficiency increase of leaves, but it could also alleviate the inhibition of PSII photochemical activity, promoting the photosynthetic electron transfer of PSII receptor side, alleviating the damage degree of OEC on PSII donor side, optimizing the energy allocation of PSII reaction center, while relatively improving the active response of a number of centers and the stability of thylakoid membrane.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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