Effect of High CO\(_2\) Concentration on Four \textit{Populus} by the Fast Fluorescence Rise OJIP

Ruyu Xie, Mu Peng, Tao Wang, Tie Li, Fanjuan Meng*

College of Life Science, Northeast Forestry University, Harbin, China

Corresponding author: Fanjuan Meng, College of Life Science, Northeast Forestry University, Harbin 150040, China. Tel: +8618845897145; Email: mfj19751@163.com


Received Date: 15 September, 2018; Accepted Date: 26 September, 2018; Published Date: 04 October, 2018

Abstract

Increased atmospheric carbon dioxide (CO\(_2\)) concentration affects plant physiological and ecosystem processes. The aim of this study was finding out the main reasons why PSII were affected by CO\(_2\) in four different kinds of \textit{Populus} (\textit{Populus} L.) (\textit{Populus} \textit{X}, \textit{Populus deltoides} × \textit{cathayana}, \textit{Populus alba} ‘Berdinensis’L., \textit{Populus euramerican} ‘N3016’ × \textit{Populus ussuriensis}) and the differences of responses in these four kinds of \textit{Populus}. The chlorophyll fluorescence technique was considered as an effective tool in the context of non-destructive leaf photosynthetic apparatus of the degree of thermal, and has been widely used in relevant studies of CO\(_2\) stress. The JIP-test is a method for analyze the fluorescence transient which transformed the measured value to a serials biological parameters, and also detect the energy strength generated by PSII. The results show that the PSII performance was negatively influenced by CO\(_2\) stress. CO\(_2\) stress resulted in down-regulation of \(\phi_{Fv}/\phi_{Po}\) and \(P_{\text{max}}\) in all four kinds of \textit{Populus}. And a significant decrease in the P-step level of the fluorescence transients OJIP curves of four \textit{Populus} species after 7 days of treatment with high CO\(_2\). So a fast decrease of the P-step level indicated there was main change to fluorescence transients. Generally speaking, these results indicated that the main reasons why PSII were affected by CO\(_2\) were degradation of antenna pigment and inhibition of the electron transport at the acceptor side of PSII.

Keywords: AtmosphericCarbonDioxide(CO\(_2\)); Photosynthetic Performance; \textit{Populus} L.; OJIP

Introduction

Environmental changes caused by increased emissions of greenhouse gasses have influenced the stability of ecosystems worldwide[1]. The increase of atmospheric carbon dioxide (CO\(_2\)) is one of the most important environmental changes in the world in the past and the future. Especially, human activities also increased the concentration of CO\(_2\) in the atmosphere, which is expected to reach 700 \(\mu\)L/L by the middle of the next century [2-5]. Current evidence showed the increase of CO\(_2\) concentration will influence on plant growth, development biological yield. In general, high CO\(_2\) concentrations increase photosynthetic rates, which promoted the growth of plants.

However, there are most reports on the response of crop plant species to elevated atmospheric CO\(_2\), but few of them have given account of the response of woody species to high CO\(_2\) levels. In this study, we compared the effect of elevated CO\(_2\) on the growth and photosynthetic characteristics of four \textit{Populus} (\textit{Populus} L.) species.

\textit{Populus} \textit{spp.}, as poplar hybrids, belongs to a fastest-growing tree. For their rapid growth, their wood can be used for construction, pulp, paper, and as a renewable, cost-effective alternative to fossil fuels. Previous reports showed that poplars are sensitive to environmental stress compared with other tree specise [6,7]. To date, the effects of high CO\(_2\) levels on \textit{Populus} physiology and growth have been studied in numerous researches. For example, in birch, elevated CO\(_2\) treatments had no major impacts on wood anatomy or wood density [8]. Lee et al., (2014) found that the cuttings of \textit{Populus alba} × \textit{glandulosa} under the elevated CO\(_2\) treatment showed reduced tree height and photosynthetic pigment contents such as chlorophyll and carotenoid. In particular, the elevated treatment resulted in a marked reduction in the chlorophyll a. However, some works also showed that increased CO\(_2\) delayed leaf fall, but this effect is species-specific. However, there are few tests of whether differences in photosynthetic ability exist between different \textit{Populus} species in response to elevated CO\(_2\). Generally,
the photosynthetic ability was reported to a reliable indirect indicator of tree performances under different environmental stress [9,10].

As a rapid and non-destructive technique, this technology is very useful to investigate the photosynthetic function, and has been used to study the effects of drought, high temperature, salt stress on plant species. Additionally, this technology has also provided valuable information on the functional and structural attributes of components involved in photosynthetic electron transport and especially to study photosystem II (PSII) behavior [11-21]. In general, a sequence of phases (labelled as O, K, J, I, P) can be obtained in the fluorescence rise during the first second of illumination from the initial \( F_0 \) to the maximal \( F_{m} \) fluorescence value. This mathematical model was named as JIP-test, which can provide quantum yields, biophysical parameters and probabilities characterizing structure and function of PSII [22,23]. PSII is very sensitive to environmental stresses [9,10]. However, a complex study comparing influences of high CO\(_2\) levels on PSII behaviors of woody species is still lack. Therefore, a detailed comparison of high CO\(_2\)-induced changes in PSII photochemistry in different Populus spp. species was carried out by using the fast Chl fluorescence records.

In our study, we have examined photosynthetic responses of four Populus spp. species to high CO\(_2\) levels using fast Chlorophyll (Chl) a fluorescence transient (O-J-I-P) technology. In this study, we analyzed the transient fluorescence using JIP-test in four Populus species (Populus X, Populus deltoides × cathayana, Populus alba 'Berdinensis ' L, Populus euramericana 'N3016' × Populus ussuriensis) under atmospheric CO\(_2\) (1500 ± 50 μmol/mol). Our objectives were to determine that whether different Populus species vary in their PSII traits under elevated atmospheric CO\(_2\).

### Materials and Methods

#### Plant Material and Stress Treatment

Experiments were performed at College of Life Science, Northeast Forestry University, which is located in Harbin, Heilongjiang Province. Four Populus species including Populus X, Populus deltoides × cathayana, Populus alba 'Berdinensis' L and Populus euramericana 'N3016' × Populus ussuriensis were used in the experiments.

The cuttings were planted in plastic pots (60 cm in length, 25 cm in breadth and 15 cm in depth) filled with 1.5 kg of soil and sand (2: 1). The experiments were carried out in the close gas-exchange system consisting of chambers of 300 L volume (71cm in length, 73cm in breadth and 175 cm in height). There are 10 cuttings in each pot. Potted plants were grown in the conditions: day/night air temperature, 28/22°C; photoperiod, 12h; relative humidity, 65-85%. One Chamber was kept at a CO\(_2\) concentration (average ± SD) of 370 ± 15 μmol/mol (control), while another chamber was treated with an elevated CO\(_2\) concentration of 1500 ± 50 μmol/mol (hereafter denoted as treatment). In order to study the effect of CO\(_2\) for every variety of poplar, we choose the seventh day after treatment to JIP-test in this study. We repeated the experiment three times with 10 plants in total, 10 for controls and 10 for elevated treatment.

#### Chlorophyll a Fluorescence Transient Measurement and the JIP-Test

JIP-test is a method to analyze the transient fluorescence, also known as fluorescence fast dynamic research method. Measuring the curve of rising fluorspar light (FLR) (rapid fluorescence kinetics) needed very strict about equipment, and the light source for high strength of saturated excitation light (strong degrees in 3000 ~ 10000 μmol photons/ (m\(^2\)s)), starting fast, and can quickly capture the fluorescence signal.

JIP-test is an important method of analysis of fluorescence kinetics curve, the purpose of this method is to turn the parameters of the measured values into a biological significance that can direct energy intensity produced by PSII. In general, there are four important inflection point on the fluorescence fast dynamic curve, namely the O - J - I - P turning point, respectively is: 1) O point shows that the system (PSI) release amount of the fluorescent light, which can be understood as the light of the PSI and efficiency, the fluorescence intensity is at 0.02 ms \( F_{o} \). 2) Fluorescence intensity at 2ms, called \( F_{i} \) inflection point reflect the reaction in the accumulation of the electron acceptor \( Q_{A} \) to \( Q_{B} \), the oxidation of the Q. 3) Fluorescence intensity at 30ms called \( F_{m} \). 4) Maximum fluorescence intensity is \( F_{m}^{\prime} \).

Leaves were dark adapted for 30 min to ensure that all PSII RCs were in the dark adapted state with open RCs. Then chlorophyll-a fluorescence transients were recorded and digitized with the portable Handy PEA (Hansatech Instruments, Ltd., King’s Lynn Norfolk, UK) with a 12-bit resolution from 10 s to 1 s and a time resolution of 10 s for the first 200 data points (Strasser et al., 1995). All measured and derived parameters (Table 1) are based on Strasser et al. [24,25] and Tsimilli-Michael[26].

---


ISSN: 2638-0013
Measured parameters from the chlorophyll-α fluorescence transient

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_t$</td>
<td>Fluorescence intensity at time measured after onset of actinic illumination</td>
</tr>
<tr>
<td>$F_o$</td>
<td>Minimum reliable fluorescence intensity at 20μs</td>
</tr>
<tr>
<td>$F_i$</td>
<td>Fluorescence intensity at the L-step (100 μs)</td>
</tr>
<tr>
<td>$F_j$</td>
<td>Fluorescence at the K-step (300 μs)</td>
</tr>
<tr>
<td>$F_k$</td>
<td>Fluorescence at the J-step (2 ms)</td>
</tr>
<tr>
<td>$F_l$</td>
<td>Fluorescence at the I-step (30 ms)</td>
</tr>
<tr>
<td>$F_p$</td>
<td>Maximum fluorescence intensity at the P-step</td>
</tr>
</tbody>
</table>

Derived parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Formula</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_j$</td>
<td>$(F_{2\text{ms}} - F_o)/(F_m - F_o)$</td>
<td>Relative variable fluorescence at the J-step</td>
</tr>
<tr>
<td>$V_i$</td>
<td>$(F_{30\text{ms}} - F_o)/(F_m - F_o)$</td>
<td>Relative variable fluorescence at the I-step</td>
</tr>
<tr>
<td>$M_o$</td>
<td>$4 \times (F_{300\text{s}} - F_o)/(F_m - F_o)$</td>
<td>Approximated initial slope per ms of the fluorescence transient</td>
</tr>
<tr>
<td>$\text{ABS/RC} = [M_o/V_j] \times [1/(F_v/F_m)]$</td>
<td>Measure of the average total absorbance per active PSII RC</td>
<td></td>
</tr>
</tbody>
</table>

Quantum yields

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Formula</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\phi_{po}$</td>
<td>$\text{TR}_o/\text{ABS} = (F_m - F_o)/F_m = F/F_m$</td>
<td>Maximum quantum yield of primary photochemistry, equal to the efficiency by which an absorbed photon trapped by the PSII RC will result in reduction of $Q_A$ to $Q_A^-$</td>
</tr>
<tr>
<td>$\psi_{Eo}$</td>
<td>$\text{ET}_o/\text{TR}_o = (1 - V)$</td>
<td>The efficiency by which a trapped exciton, having triggered the reduction of $Q_A$ to $Q_A^-$ can move an electron further than $Q_A^-$ into the intersystem electron transport chain</td>
</tr>
<tr>
<td>$\delta_{Ro}$</td>
<td>$\text{RE}_o/\text{ET}_o = (1 - V)/(1 - V)$</td>
<td>The efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI end acceptors</td>
</tr>
</tbody>
</table>

Performance index

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Formula</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{I_{\text{abs}}}$</td>
<td>$[\text{RC}/\text{ABS}] \times [\phi_{po}/(1 - \phi_{po})] \times [\psi_{Eo}/(1 - \psi_{Eo})]$</td>
<td>Performance index on absorption basis, incorporating the steps from antenna, reaction center and electron transport parameters</td>
</tr>
<tr>
<td>$P_{I_{\text{total}}}$</td>
<td>$[\text{RC}/\text{ABS}] \times [\phi_{po}/(1 - \phi_{po})] \times [\psi_{Eo}/(1 - \psi_{Eo})] \times [\delta_{Ro}/(1 - \delta_{Ro})]$</td>
<td>Total performance index per absorption basis, integrates into the sum of changes in quantum yields and absorption</td>
</tr>
</tbody>
</table>

Table 1: Formulae and explanation the technical data of the OJIP curves and the selected JIP-test parameters used in this study.
Poplar leaves can completely cover the fluorescent clip test hole, it can measure directly. The excitation light was red light whose intensity was 3000 µmol/m² s saturated light intensity with peak wavelength of 650 nm. Fluorescent signal recording time of 10s, each group of plant test repeat 5 times.

**Date Analysis**

Each experiment was conducted at least five times independently. All data presented were mean values of each treatment. Standard Errors (SE) for the values obtained were calculated. All the above data analyses were processed using SPSS17.0 Software.

**Results**

### Changes in Photosystem II (PS II) Under CO₂ Stress

In Figure 1, four typical O-J-I-P chlorophyll fluorescence transient in leaves of untreated and treated seedlings of four *Populus* species are shown. To analyze changes in the shape of the transients among all *Populus* genotypes, All O-J-I-P transients were normalized at the O- and P-step. In four genotypes the response of treated seedlings was not similar.

In *Populus X*, at the 7th day, relative fluorescence intensity was decreased (Figure 1A). Thus high CO₂ conditions after long time inhibited the fluorescence intensity, especially from I- to P- step of *Populus X*. In *Populus deltoides × cathayana*, at 7th day, relative fluorescence intensity was increased. While, relative fluorescence intensity was increased from J- to I- step (Figure 1B), which showed different changes from these genotypes in relative fluorescence intensity. In *Populus alba ‘Berdinensis’* L, relative fluorescence intensity was similar between control and treatment from J- to I- step (Figure 1C). In *Populus euramericana ‘N3016’ × Populus ussuriensis*, an increase in relative fluorescence intensity was observed from I- to P- step (Figure 1D).

In parallel, all *Populus* species showed significant decrease in maximum quantum yield of PSII primary photochemistry ($\phi_{Po}$), the efficiency with which the energy of a trapped exciton is converted into electron transport beyond QA- ($\psi_{Eo}$), the quantum yield of electron transport beyond QA (\$\phi_{Eo}$) and total performance index per absorption basis ($PI_{total}$) after high CO₂ treatment. Especially, $PI_{total}$ value sharply decreased after treatment. On the other hand, the values of relative variable fluorescence at the J-step ($V_{j}$) and relative variable fluorescence at the I-step ($V_{i}$) of treated-plants were higher than those of controls. There is higher value in ABS/RC (measure of the average total absorbance per active PSII RC) of treated plants compared to those of control plants. However, an exception for the *Populus deltoides × cathayana* after treatment had no difference between CO₂-treated and control plants (Table 2).

### Changes of Fluorescence Parameters Under CO₂ Stress

In Table 2, no significant high CO₂-induced changes were observed in the minimum fluorescence ($F_{o}$), fluorescence at the J-step ($F_{j}$) and fluorescence at the I-step ($F_{i}$). In contrast, drastic decreases in $F_{m}$ maximum fluorescence ($F_{m}$) values of four *Populus* species were recorded after 7 days of CO₂ treatment (Table 2). In parallel, all *Populus* species showed significant decrease in maximum quantum yield of PSII primary photochemistry ($\phi_{Po}$), total performance index per absorption basis ($PI_{total}$) after high CO₂ treatment. Especially, $PI_{total}$ value sharply decreased after treatment. On the other hand, the values of relative variable fluorescence at the J-step ($V_{j}$) and relative variable fluorescence at the I-step ($V_{i}$) of treated-plants were higher than those of controls. There is higher value in ABS/RC (measure of the average total absorbance per active PSII RC) of treated plants compared to those of control plants. However, an exception for the *Populus deltoides × cathayana* after treatment had no difference between CO₂-treated and control plants (Table 2).

<table>
<thead>
<tr>
<th>Species</th>
<th><em>Populus X</em></th>
<th><em>Populus deltoides × cathayana</em></th>
<th><em>Populus alba ‘Berdinensis’</em> L.</th>
<th><em>Populus euramericana ‘N3016’ × Populus ussuriensis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Measured parameters</td>
<td>Control</td>
<td>Treatment</td>
<td>Control</td>
<td>Treatment</td>
</tr>
<tr>
<td>$F_{o}$</td>
<td>2798±171 .57</td>
<td>2345±166.19</td>
<td>2576.75±116.56</td>
<td>2643.6±96.32</td>
</tr>
<tr>
<td>$F_{m}$</td>
<td>2284.4±75.49</td>
<td>2065±98.63</td>
<td>2282.75±66.48</td>
<td>2298.25±280.5</td>
</tr>
</tbody>
</table>

**Figures 1(A-D):** The relative fluorescence transients in fully dark-adapted *Populus X* leaves (A), *Populus deltoides × cathayana* leaves (B), *Populus alba ‘Berdinensis’* L leaves (C) and *Populus euramericana ‘N3016’ × Populus ussuriensis* leaves (D) under CO₂ stress and the control condition at 7 days.
Discussion

In this study, the shapes of chlorophyll a fluorescence transient (O-J-I-P) were markedly different in the four high CO$_2$-treated leaves, however the values of $F_v/F_m$ showed similar decreasing tendency (Table 2). Thus, there is the heterogeneous behavior of PSII in _Populus_ leaves as similar $F_v/F_m$. Generally, the level of photochemical reaction can be evaluated according to the chlorophyll fluorescence intensity. And $F_v/F_m$ was used to describe the trapping efficiency of the absorbed light, which can reduce primary quinone electron acceptor of PSII (Q$_A$) [27]. Therefore, the different fluorescence transients of leaves under high CO$_2$ levels indicated that the photochemical reactions were different in differ _Populus_ species, although there were similar decreasing $F_v/F_m$ (reflecting trapping efficiencies of the absorbed light). In contrast, previous studies showed the $F_v/F_m$ ratio reflects the photochemical efficiency of PSII [28]. Various environmental stress can lead to inhibition of photosynthetic efficiency, accordingly, affecting state...
of the photosynthetic apparatus.

For O-J-I-P, point O represents the fluorescence of PS II action center when all of the electron acceptor (Q_A, A^m, PQ, etc.) are fully open in the maximum oxidation state. The fluorescence intensity of point O is connected with the content of the antenna pigment and the activity of action center. Point J reflects the rate of reduction of Q_A, which is connected with reaction centre pigments, light-harvesting pigment and the state of Q_A and Q_A^m. If the electrons transfer from Q_A to Q_B is restricted, the value of point J will rise [29]. Here, higher J value in the leaves. In addition, point I reflects the heterogeneity of PQ. The electron acceptor state in point I mainly is related to Q_A and Q_A^m. Furthermore, the structure and function of PS II complexes and the size of the PQ library is attributed to appearing time of point P. In this study, high CO_2 level caused a significant decrease in the P-step level of the fluorescence transients OJIP curves of four Populus species after 7 days of treatment with high CO_2. So a fast decrease of the P-step level indicated there was main change to fluorescence transients. Generally, the “P” level was connected with the process of the electron transportation from Q_A to PQ, and it can mark concentrations of both Q_A and PQ[30, 31].

No significant changes in F_v, F_m and F_i of different Populus species were observed when subjected to high CO_2 stress. However, high CO_2 stress result F_m in decrease. F_m reflects maximum fluorescence intensity at the P-step[32]. Therefore, this result suggested that the F_m may be a good marker of PSII vitality under CO_2 stress.

The PSII switches from the process of converting light energy into biochemical energy storage to the energy conversion process that transforms absorbed light energy into heat dissipation [33]. One of parameters used to analyze the response of the plant’s PSII is PI_total, PI_total is sensitive to changes in either antenna properties, trapping efficiency or electron transport beyond Q_A [34]. In this study, we found that PSII performance was represented using this index (PI_total). In general, the observed PI_total is influenced by changes in antenna, RC, electron transport and end-acceptor reduction dependent parameters. Thus, PI_total integrates the response of RC/ABS, TRo/ABS(=F_i/F_m), ETo/TRo (= [1 – V_j]) and REo/ETo(=[1 – V_j]/[1 – V_j]).

In summary, inhibition of PSII under CO_2 stress involved in changes of F_v and PI_total. In other words, these two parameters can be used in indicate the changes of PSII. These results indicated that the main reasons why PSII were affected by CO_2 were degradation of antenna pigment and inhibition of the electron transport at the acceptor side of PSII.

Acknowledgement

This study was supported by the Fundamental Research Funds for the Central Universities (No. 2572015DA03 and No. 2572016EAI4).

Conflict of Interest

The authors declare that there are no conflicts of interest.

References

1. IPCC (2007) Intergovernmental panel on climate change, Climatic change 2007: the physical science basis, Geneva.


