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# Interactive effects of elevated $CO_2$ and drought on photosynthetic capacity and PSII performance in maize

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## Abstract

Elevated atmospheric $CO_2$ concentration [$CO_2$] and the change of water distribution in arid and semiarid areas affect plant physiology and ecosystem processes. The interaction of elevated [$CO_2$] and drought results in the complex response such as changes in the energy flux of photosynthesis. The performance of photosystem (PS) II and the electron transport were evaluated by using OJIP induction curves of chlorophyll *a* fluorescence and the $P_N$-$C_i$ curves in the two-factor controlled experiment with [$CO_2$] of 380 (AC) or 750 (EC) [$\mu$mol mol$^{-1}$] and water stress by 10% polyethylene glycol 6000. Compared to water-stressed maize (*Zea mays* L.) under AC, the EC treatment combined with water stress decreased the number of active reaction centers but it increased the antenna size and the energy flux (absorbed photon flux, trapping flux, and electron transport flux) of each reaction center in PSII. Thus, the electron transport rate was enhanced, despite the indistinctively changed quantum yield of the electron transport and energy dissipation. The combination of EC and the water-stress treatment resulted in the robust carboxylation rate without elevating the saturated photosynthetic rate ($P_{max}$). This study demonstrated that maize was capable of transporting more electrons into the carboxylation reaction, but this could not be used to increase $P_{max}$ under EC.

*Additional key words*: drought stress; elevated $CO_2$ concentration; OJIP induction curves; $P_N$-$C_i$ curves.

## Introduction

Global climate changes exert remarkable influence not only on [$CO_2$], but also on global distribution of arid and semiarid areas. Interactions of carbon and water, and their influence on the global carbon cycle are of great changes may affect plant metabolism directly, such as photosynthesis and growth regulation (de Graaff *et al.*

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*Abbreviations*: ABS/$CS_M$ − absorbed photon flux per cross section; ABS/RC − average absorbed photon flux per PSII reaction center; AC – ambient [$CO_2$]; AQY − apparent quantum yield; Area − total complementary area between fluorescence induction curve and $F_M$; CE − carboxylation efficiency; Chl − chlorophyll; $C_i$ − intercellular $CO_2$ concentration; [$CO_2$] − $CO_2$ concentration; D − drought; $E$ – transpiration rate; EC – elevated [$CO_2$]; $ET_0/CS_M$ − electron transport flux from $Q_A$ to $Q_B$ per cross section; $ET_0$/RC − electron transport flux from $Q_A$ to $Q_B$ per PSII; $F_0$ − minimal fluorescence of the dark-adapted state; $F_I$ − fluorescence at I step; $F_J$ − fluorescence at J step; $F_K$ − fluorescence at K step; $F_M$ − maximal fluorescence of the dark-adapted state; $F_t$ − fluorescence at time t after onset of actinic illumination; FACE – free air $CO_2$ enrichment; $g_s$ – stomatal conductance; $J$ − electron transport rate; $M_0$ − approximate value of the initial slope of relative variable Chl fluorescence curve $V_t$; N − reduction times of $Q_A$ from $F_0$ to $F_M$; PEG − polyethylene glycol; PEPC – phospho*enol*pyruvate carboxylase; $PI_{total}$ − performance index for energy conservation from photons absorbed by PSII antenna until the reduction of PSI acceptors; $P_{max}$ – light- and $CO_2$-saturated photosynthetic rate; PPFD − photosynthetic photon flux density; PQ − plastoquinone pool; PS − photosystem; $Q_A$ − primary quinone acceptor of PSII; $Q_B$ − secondary quinone acceptor of PSII; RCs − reaction centers; RC/$CS_M$ − probability that PSII Chl functions as an active center; RH – relative humidity; Rubisco – ribulose-1,5-bisphosphate carboxylase/oxygenase; $S_M$ − normalized area; $t_0$ − time to reach $F_0$; $t_{FM}$ − time to reach maximal fluorescence $F_M$; $TR_0/CS_M$ − maximum trapped exciton flux per cross section; $TR_0$/RC − maximum trapped exciton flux per PSII; $\varphi_{D0}$ − quantum yield (at $t_0$) for energy dissipation; $\varphi_{E0}$ − quantum yield for electron transport; $\varphi_{Pav}$ − average (from $t_0$ to $t_{FM}$) quantum yield for primary photochemistry; $\varphi_{P0}$ − maximum quantum yield of primary PSII photochemistry; $\psi_0$ − probability (at $t_0$) that the trapped exciton moves an electron into the electron transport chain beyond $Q_A^-$; $\delta_{R0}$ − the efficiency of electron movement from the reduced intersystem electron acceptors to the PSI end acceptors.

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2006) importance to atmosphere-biosphere interactions and human society (Reich *et al.* 2006). Some climate driven. Analyses of the [$CO_2$] effect and its interaction with other environmental factors are of great relevancy since the responsiveness of plants to EC differs with water availability (Prins *et al.* 2011).

Photosynthetic acclimation caused by EC has been reported variously (Gutiérrez *et al.* 2009, Martínez-Carrasco *et al.* 2005, Sarker and Hara 2011). Experiments conducted under both controlled and field conditions in free air $CO_2$ enrichment (FACE) system have shown that $C_3$ plants often maintain photosynthesis during short-term drought under EC due to the improved water use efficiency, however, it reduces long-term adaption and results in downregulation of photosynthesis (Ainsworth and Rogers 2007, Albert *et al.* 2011, Long *et al.* 2004). Experiments showed that $C_4$ plants are less sensitive than $C_3$ plants to EC (Ghannoum *et al.* 2011, Sage and Kubien 2003, Wand *et al.* 1999). The $C_4$ plants are known to have $CO_2$-concentrating mechanism for two types of photosynthetic cells, the mesophyll and bundle sheath cells (Ghannoum *et al.* 2011). $CO_2$ is initially fixed by phospho*enol*pyruvate carboxylase (PEPC) in the mesophyll cells into $C_4$ acids which then diffuse to the bundle sheaths where they are decarboxylated to supply $CO_2$ for ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Ghannoum *et al.* 2011). Thus, the $C_4$ plants possess near saturating photosynthesis at current AC (Ghannoum *et al.* 2011, 2000). Therefore, the rise in atmospheric [$CO_2$] could have theoretically only the small direct effect on $C_4$ photosynthesis. And besides, the photosynthesis acclimation of $C_3$ plants is often accompanied by reduction in foliar N (Stitt 1991, Zhou and Shangguan 2009). In $C_4$ leaves, photosynthesis could reach the maximum level with lesser amount of Rubisco protein under both current and enriched [$CO_2$] by the $CO_2$-concentrating mechanism (Furbank *et al.* 1996, Ghannoum *et al.* 2000). Thus, the reduction in foliar N could theoretically exert less negative impact on $C_4$ plants in the $CO_2$-enriched environment. Moreover, the $C_4$ plants were observed to have the fast growth rate under EC. Since the $C_4$ plants have almost saturated photosynthesis under AC, the fast growth rate was thought to be related to the number of potential mechanisms (Ghannoum *et al.* 2000, Grodzinski *et al.* 1998).

Data from previous studies revealed that $C_4$ plants growth at enhanced [$CO_2$] alleviates the negative effects of drought stress on photosynthesis by stomata acclimation (Markelz *et al.* 2011, Vu and Allen 2009), which reduces stomatal conductance ($g_s$) and transpiration ($E$) with no significant change in the net photosynthetic rate ($P_N$) (Ghannoum *et al.* 2011). However, the electron flux in the PSII of $C_4$ plants under EC and drought stress remains poorly understood but plays the important role in photosynthesis. Chlorophyll (Chl) *a* fluorescence transients can detect subtle variations in photosynthesis (Strasser *et al.* 2010) and they present energy fluxes from absorption in the Chl antenna to the electron transport (Strasser *et al.* 2004, Strasser *et al.* 2010, Tsimilli-Michael and Strasser 2008). The energy flux includes photon-flux absorption and the excitation of Chl in antennal pigments, called the absorption flux. The excitation energy, caused by excited Chl, is quenched in two ways. One way dissipates the energy as heat or fluorescence emission; the other way channels the energy to the reaction center, it is called the trapping flux. The trapping flux converts into the redox energy by reducing the electron acceptor $Q_A$ to $Q_A^-$ that is then reoxidized to $Q_A$ reducing the electron transport chain beyond $Q_A^-$ (called the electron transport flux) and ultimately leading to $CO_2$ fixation (Strasser and Strasser 1995, Strasser *et al.* 2004). These processes produce NADPH and ATP by accumulating protons in the thylakoids (Taiz and Zeiger 2006). These fluxes can be expressed per the fully active PSII reaction center (specific energy fluxes) and per the excited cross section (phenomenological energy fluxes) of the photosynthetic sample (Strasser *et al.* 2004). Understanding this process is essential to explore photosynthetic regulations in a future climate. The OJIP fluorescence curve is highly useful to describe these fluxes, starting at O level to P, the maximum point, and going usually through the series of transient curve inflections, namely J and I (Lazár 2009). The relative fluorescence variations during the phases O–J, J–I, and I–P are evaluated from the difference between the initial fluorescence and the fluorescence at the different steps. The J point corresponds to the peak of [$Q_A^-\,Q_B$] and [$Q_A^-\,Q_B^-$]. The O–J phase mainly represents primary photochemistry, including the reduction of the primary electron acceptor in PSII (pheophytin) and of the first quinone electron acceptor of PSII ($Q_A$) (Lazár 2009, Lazár *et al.* 1997, Stirbet *et al.* 1998, Strasser and Strasser 1995, Strasser *et al.* 2004); the I point often corresponds to the first shoulder of the [$Q_A^-\,Q_B^{2-}$]; and the P point to the maximum concentrations of [$Q_A^-\,Q_B^{2-}$] and [$PQH_2$] (Lazár 2006, Zhu *et al.* 2005). The I–P phase should indicate the accumulation of double reduced $Q_B$ and the reduced plastoquinone pool molecules (Zhu *et al.* 2005). Thus, the OJIP transients reflect the PSII performance. Relative parameters can be calculated with different formulas to evaluate the PSII performance and the electron fluxes (Strasser *et al.* 2004).

This paper presents an attempt to clarify the responses of Chl fluorescence transients in response to drought and EC. We used the technique of the fast fluorescence OJIP curve and $P_N$–$C_i$ curve to explore the carboxylation capability and PSII performance in maize under EC and drought. The results may help to understand the mechanism of photosynthesis adaptation in maize under EC.

## Materials and methods

**Plant material and growth conditions**: Maize (*Zea mays* L. cv. Zhengdan 958) seeds were obtained from Northwest Agriculture and Forestry University (Yangling, Shaanxi, China). The seeds were disinfected using 20% (w/v) NaClO for 30 min to prevent fungal infection, rinsed with distilled water, then placed on the moist filter paper for germination in a dark chamber at 30ºC for about 2 d. After emergence, the seedlings were grown hydroponically in two closed climate chambers (*AGC–D001P*, *Qiushi Corp*., China), which were controlled at 11 h - dark [18ºC, relative humidity (RH) of 50%]/ 13 h - light regime (25ºC, RH 40%, 300 [μmol(photons) $m^{-2}$ $s^{-1}$]) from 7:30 to 20:30 h. The plants were fertilized by 1/2 strength modified Hoagland nutrient solution (Taiz and Zeiger 2006) and aerated using aquarium diffusers.

When the seedlings were grown for 2 d and the first leaf expanded more than 80%, two closed climate chambers were controlled under AC or EC until the study was completed. Half of the 12-d-old plants with three fully expanded leaves were transplanted to 1/2 Hoagland solutions containing 10% PEG 6000 (–0.2 MPa) and grown further for 15 d. Another half of plants continued to grow in 1/2 Hoagland nutrient solutions without PEG treatment (control).

The experimental design was a randomized completely block design with eight replications. They were treated in the following way:

| Treatment | |
|---|---|
| Control | AC × well watered |
| D | AC × 10% PEG |
| C750 | EC × well watered |
| C750×D | EC × 10% PEG |

**Analysis of Chl *a* fluorescence transients and ETR**: Noninvasive Chl *a* fluorescence measurements were performed with the portable *Handy PEA* (*Hansatech Instruments, Ltd*., King's Lynn Norfolk, UK). Leaves were dark-adapted for 20 min to ensure that all PSII RCs were open. Then Chl *a* fluorescence transients were recorded and digitized with a 12-bit resolution from $1 \times 10^{-5}$ s to 1 s and a time resolution of $1 \times 10^{-5}$ s for the first 200 data points (Strasser and Strasser 1995). The excitation light intensity was 600 W $m^{-2}$ from the array of six red light emitting diodes with the peak wavelength of 650 nm focused on the leaf surface on a spot of 4 mm in diameter. The Chl *a* fluorescence signals were detected by a PIN photocell after passing through a long pass filter (50% transmission at 720 nm) (Srivastava *et al.* 1997). All measured and derived parameters were based on Strasser *et al.* (2004, 2010) and Tsimilli-Michael and Strasser (2008).

On the logarithmic time scale, the rising transient from $F_0$ (F measured at $5 \times 10^{-5}$ s, when all the RCs of PSII were open, *i.e.*, when $Q_A$ was fully oxidized) to $F_P$ (where $F_P = F_M$ under the saturating excitation light, of which the excitation intensity was high enough to ensure the closure of all the RCs of PSII, *i.e.*, the full reduction of all the RCs) showed the polyphasic behavior. The analysis of the transient took into consideration fluorescence values at $5 \times 10^{-5}$ s ($F_0$, step O), $1 \times 10^{-4}$ s ($F_{0.0001\ s}$), $3 \times 10^{-4}$ s ($F_{0.0003\ s}$, step K), $2 \times 10^{-3}$ s ($F_{0.002\ s}$, step J), $3 \times 10^{-2}$ s ($F_{0.03\ s}$, step I), and the maximal level ($F_M$, step P). The OJIP test represents a translation of original data into biophysical parameters that quantify the energy fluxes through PSII. The equations and definitions of OJIP parameters are shown in Table 1.

**Analysis of $P_N$-$C_i$ curve**: The youngest, fully expanded leaves were chosen for measurements at 9:00 and 11:00 h (local time). The measurements were repeated 3 to 5 times (one leaf from one replicate plant) per the treatment. The photosynthetic measurements were carried out with the same leaves over time to avoid any additional variations. $P_N$, photosynthetic responses to intercellular $CO_2$ concentration ($C_i$), and the photosynthetic photon flux density (PPFD) were determined with *Li-6400 Portable Photosynthesis System* (*Li-Cor*, Lincoln, USA). Under AC or EC, $P_N$ was measured at 300 μmol $m^{-2}$ $s^{-1}$ PPFD. The $P_N$-PPFD curves were measured at 2,000; 1,800; 1,600; 1,400; 1,200; 1,000; 800, 600, 400, 300, 250, 200, 150, 100, 80, 50, 20, and 0 μmol(photon) $m^{-2}$ $s^{-1}$ PPFD. The apparent quantum yield (AQY) and saturated PPFD were derived from the $P_N$-PPFD curve. Under saturated PPFD, $P_N$ was measured at 2,200; 2,000; 1,800; 1,600; 1,400; 1,200; 1,000; 800, 600, 400, 300, 250, 200, 150, 100, 80, and 50 μmol($CO_2$) $mol^{-1}$ in the reference chamber. RH of the air in the leaf chamber was controlled at 35%, and the leaf temperature at 22°C. $P_N$ was recorded when the sample leaf was balanced for 100 s under each PPFD and 300 s in each [$CO_2$] step. Photosynthesis measured at PPFD of 1,800 μmol $m^{-2}$ $s^{-1}$ and [$CO_2$] of 2,000 μmol $mol^{-1}$ was determined as the light and the $CO_2$-saturated photosynthetic rate in this study. Prior to the measurement, the leaf sample was illuminated with saturated PPFD provided by the light emitting diodes of the equipment for 600−1,200 s. No significant photoinhibition occurred during the measurements. Calculation of the PEPC carboxylation efficiency (CE), the $CO_2$-saturated photosynthetic rate ($P_{max}$), and the electron transport rate (*J*) followed the model of $C_4$ photosynthesis developed by von Caemmerer and Furbank (1999).

Table 1. Equations and definitions of OJIP parameters (based on information presented by Strasser and coworkers). For reasons of clarity, several notations and definitions of OJIP parameters used by Strasser and collaborators have been modified (for equivalence of notation) (Strasser and Strasser 1995, Strasser *et al.* 2010, Tsimilli-Michael and Strasser 2008).

| Information selected from the fast OJIP fluorescence induction (data necessary for the calculation of the so called OJIP parameters) | |
|---|---|
| Data extracted from the recorded fluorescence transient OJIP | |
| $F_0$ | Minimal fluorescence of dark adapted state |
| $F_M$ | Maximal fluorescence of dark adapted state |
| $F_t$ | Fluorescence at time t after onset of actinic illumination |
| $F_K = F_{0.0003\,s}$ | Fluorescence at K step of OJIP |
| $F_J = F_{0.002\,s}$ | Fluorescence at J step of OJIP |
| $F_I = F_{0.03\,s}$ | Fluorescence at I step of OJIP |
| Variable fluorescence | |
| $V_K = (F_K - F_0)/(F_M - F_0)$ | Relative variable Chl fluorescence at K-level |
| $V_J = (F_J - F_0)/(F_M - F_0)$ | Relative variable Chl fluorescence at J-level |
| $V_I = (F_I - F_0)/(F_M - F_0)$ | Relative variable Chl fluorescence at I-level |
| Basic parameters for PSII | |
| $M_0 = 4\,(F_{0.0003\,s} - F_0)/(F_M - F_0)$ | Approximate value of the initial slope of relative variable Chl fluorescence curve $V_t$ |
| $S_M = (\text{Area})/(F_M - F_0)$ | Normalized area (assumed proportional to the number of reduction and oxidation of one $Q_A^-$ molecule during the fast OJIP transient, and therefore related to the number of electron carriers per electron transport chain) |
| $N = S_M \times M_0 \times (1/V_J)$ | Reduction times of $Q_A$ from $F_0$ to $F_M$ |
| $RC/CS = \varphi_{P0}\,(V_J/M_0)\,(ABS/CS_M)$ | Probability that a PSII Chl function as active centers |
| $PI_{total} = \frac{RC}{ABS} \times \frac{\varphi_{P0}}{1-\varphi_{P0}} \times \frac{\psi_0}{1-\psi_0} \times \frac{\delta_{R0}}{1-\delta_{R0}}$ | Performance index for energy conservation from photons absorbed by PSII antenna, until the reduction of PSI acceptors |
| Yields and flux ratios | |
| $\varphi_{P0} = 1 - F_0/F_M$ | Maximum quantum yield of primary PSII photochemistry |
| $\psi_0 = (1 - V_J)$ | Probability (at time 0) that a trapped excition moves an electron into the electron transport chain beyond $Q_A^-$ |
| $\varphi_{E0} = [1 - F_0/F_M]\,\psi_0$ | Quantum yield for electron transport |
| $\varphi_{Pav} = \varphi_{P0}\,(1 - V_J) = \varphi_{P0}\,(S_M/t_{FM})$ | Average (from time 0 to $t_{FM}$) quantum yield for primary photochemistry |
| $\varphi_{D0} = 1 - \varphi_{P0}$ | Quantum yield (at t = 0) for energy dissipation |
| $\delta_{R0} = (1-V_I)/(1-V_J)$ | The efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI end acceptors |
| Specific energy fluxes (per active PSII reaction center) | |
| $ABS/RC = (M_0/V_j) \times (1/\varphi_{P0})$ | Average absorbed photon flux per PSII reaction section center |
| $TR_0/RC = M_0/V_j$ | Maximum trapped excition flux per PSII |
| $ET_0/RC = (M_0/V_j) \times \psi_0$ | Electron transport flux from $Q_A$ to $Q_B$ per PSII |
| Phenomenological energy fluxes/activities (per excited cross section CS) | |
| $ABS/CS_M = F_M$ | Absorbed photon flux per cross section |
| $TR_0/CS_M = \varphi_{P0}\,(ABS/CS_M)$ | Maximum trapped excition flux per cross section |
| $ET_0/CS_M = \varphi_{E0}\,(ABS/CS_M)$ | Electron transport flux from $Q_A$ to $Q_B$ per cross section |

**Statistical analysis**: Data from the independent experiments presented in figures are in the form of means ± SD. Significance of differences were (at $P<0.05$) tested using *SAS for Windows V8* (*SAS Institute*, Cary, NC, USA), and the differences among the treatments were tested by *Duncan*'s multiple range test.

## Results

**PSII reaction center and quantum yield**: The shape of OJIP transient was sensitive to drought stress (Fig. 1). The performance index in Fig. 2 illustrated that treatment C750×D increased $M_0$ and N but decreased $RC/CS_M$ compared with D and C750 treatments, and the control. The electron efficiencies ($\varphi_{P0}$, $\varphi_{E0}$, $\delta_{R0}$, and $\psi_0$) of C750×D were not different from the D treatment, but they were lower than the control (decreased 2.6%, 13.5%, and 11.2%, respectively). The $\varphi_{Pav}$ of C750×D was about 64.8% of D, which could be due to the increased number of closed or silent PSII RCS in C750×D. The quantum yield (at $t_0$) for energy dissipation ($\varphi_{D0}$) of C750×D was 9.8% lower than control. C750×D and D markedly increased $V_K$, $V_J$, and $V_I$ (Table 2). The changes in $V_K$, $V_J$, $S_M$, and N in C750 did not differ from those in the control.

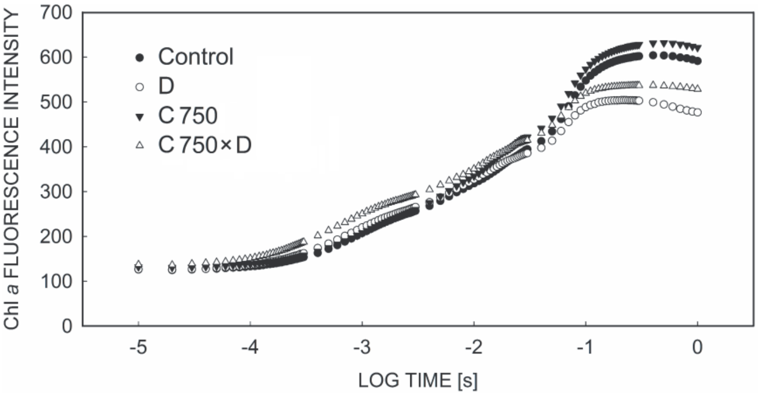

Fig. 1. The shapes of polyphasic fluorescence rise OJIP transients in the treatment with doubled $CO_2$ concentration under drought stress, plotted on a logarithmic time scale from $1\times10^{-5}$ [s] to 1 [s]. The transients were normalized on $F_0$. The legend symbols stand for the time points set in the OJIP test for calculating the structural and functional parameters in the four treatments.

**PSII energy fluxes**: The performance index in Fig. 3 illustrated that treatment C750×D increased ABS/RC, $TR_0/RC$, and $ET_0/RC$ compared with D and C750 treatments, and the control. The $ABS/CS_M$, $TR_0/CS_M$, and $ET_0/CS_M$ of C750×D were as high as the control, and about 9.3%, 11.3%, and 46.6% higher than D treatment, respectively. Analysis revealed that C750×D had the lowest $RC/CS_M$ among four treatments, but it had the highest probability of energy fluxes transit in per active PSII reaction center.

**$P_N$-$C_i$ parameters**: shows the performance index of carbon reaction under different treatments. The CE of C750 and C750×D were significantly higher than that of control and D (Fig. 4). The C750 treatment had the highest $P_{max}$, while $P_{max}$ of C750×D was not significantly higher than D. Dramatically, the *J* in C750×D reached the highest level among the four treatments, and in C750 treatment reached the second highest. Conversely, the control had the lowest level of *J*. The Fig. 4 indicates that EC helped to maintain high CE and *J*, but low $P_{max}$ in water-stressed maize.

## Discussion

EC may directly or indirectly affect the PSII performance (Maroco *et al.* 1999). Changes in stomatal conductance, electron transport efficiency, and carboxylation capacity altered consumption of the energy equivalents in the carbon reaction and the need for the controlled energy dissipation within PSII and alternative electron sinks (Lawlor and Tezara 2009). Therefore, photosynthetic capacity is affected by natural variation in ecosystem as well as by the experimental treatments. Controlled experiments could interpret precisely the combination of climatic variability.

In the current study, EC did not change $\varphi_{P0}$, $\psi_0$, and $\varphi_{E0}$, but decreased $\varphi_{Pav}$, and significantly increased *J* and CE in well watered maize compared with the control plants. The reduced $\varphi_{Pav}$ in C750 plants was caused by the reduction in $\delta_{R0}$. This indicated that C750 plants decreased the probability that an electron can move from the reduced intersystem electron acceptors to the PSI end acceptors. Thus, C750 rose the probability of energy dissipation in PSI acceptors. However, EC helped the leaves to enlarge significantly the apparent antenna size per active PSII (ABS/RC) and to enlarge the trapped exciton flux per PSII ($TR_0/RC$), which could counteract the reduction of $\delta_{R0}$ and maintain the $ABS/CS_M$, $TR_0/CS_M$, and $ET_0/CS_M$ at the same level as the control. In general, acclimation of photosynthesis reduces the energy equivalents (ATP and NADPH) consumption in the Calvin-Benson cycle, which increases the probability of overexcitation in PSII and alternative electron sinks (Lawlor and Tezara 2009, Wilhelm and Selmar 2011). In well watered plants under AC, the energy dissipation is more important in photoprotection due to the higher restriction of carboxylation (Wilhelm and Selmar 2011). In the $CO_2$-enriched environment, the significantly enhanced energy supply (*J*) could not induce $P_{max}$ downward acclimation for the robust carboxylation capacity

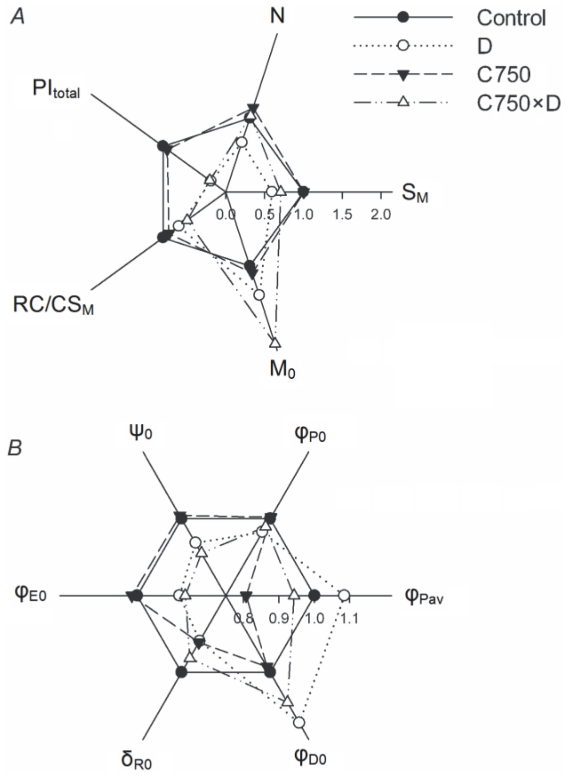

Fig. 2. Performance index changes from OJIP induction curves under different treatments. *A*: basic parameters for PSII, *B*: quantum yields and efficiencies/probabilities. The values represent the means of 8 replications. $S_M$ − normalized area; $RC/CS_M$ − probability that PSII Chls function as active centers; $PI_{total}$ − performance index for energy conservation from photons absorbed by PSII antenna, until the reduction of PSI acceptors; N − reduction times of $Q_A$ from $F_O$ to $F_M$; $M_0$ − approximate value of the initial slope of relative variable Chl fluorescence curve $V_t$; $\varphi_{D0}$ − quantum yield (at t = 0) for energy dissipation; $\varphi_{E0}$ − quantum yield for electron transport; $\varphi_{Pav}$ − average (from time 0 to $t_{FM}$) quantum yield for primary photochemistry; $\varphi_{P0}$ − maximum quantum yield of primary PSII photochemistry; $\psi_0$ − probability (at t = 0) that a trapped excition moves an electron into the electron transport chain beyond $Q_A^-$; $\delta_{R0}$ − the efficiency of electron movement from the reduced intersystem electron acceptors to the PSI end acceptors.

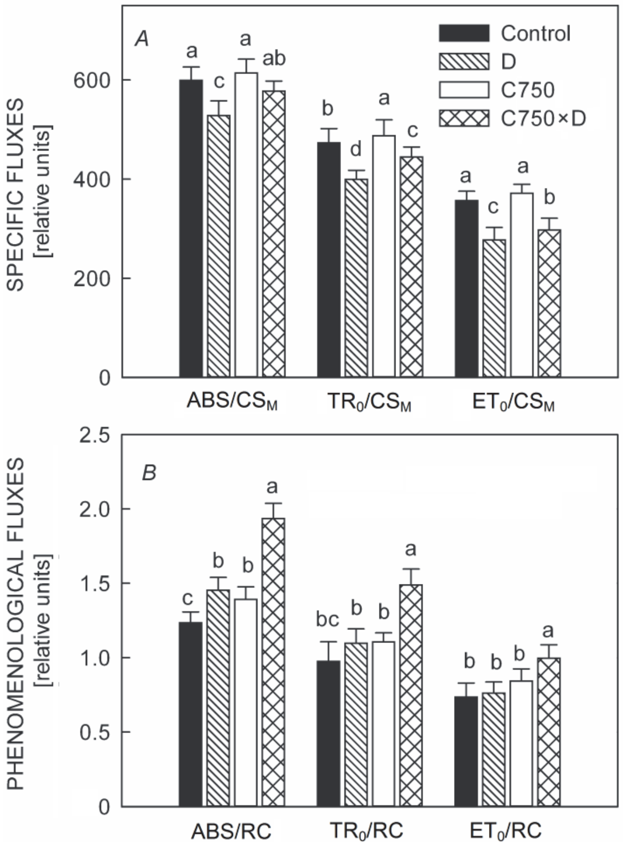

Fig. 3. Energy fluxes changes from OJIP induction curves under different treatments. *A*: phenomenological energy fluxes/ activities (per excited cross section CS); *B*: specific energy fluxes (per active PSII reaction center). The values represent the means of 8 replications and the bars stand for SE. *Different letters* indicate statistical difference significance at $P<0.05$ among the treatments by *Duncan*'s multiple range test. $ABS/CS_M$ − absorbed photon flux per cross section; ABS/RC − average absorbed photon flux per PSII reaction section center; $ET_0/CS_M$ − electron transport flux from $Q_A$ to $Q_B$ per cross section; $ET_0/RC$ − electron transport flux from $Q_A$ to $Q_B$ per PSII; $TR_0/CS_M$ − maximum trapped excition flux per cross section; $TR_0/RC$ − maximum trapped excition flux per PSII;

(Ghannoum *et al.* 2000). These response patterns indicated that the well watered maize seedlings were capable of maintaining $P_{max}$ in the $CO_2$-enriched environment.

Drought is often reported to restrain PSII performance by increasing energy dissipation if the energy transported from PSII excesses consumption capacity in Calvin-Benson cycle under AC (Guan and Gu 2009, Jin *et al.* 2008, Wilhelm and Selmar 2011). Results of the current study were in agreement with previous studies (Albert *et al.* 2011, Oukarroum *et al.* 2009) that the D plants reduced $PI_{total}$, $\varphi_{E0}$, $\psi_0$, and $\delta_{R0}$ but increased $\varphi_{D0}$. C750×D did not increase $\varphi_{E0}$ and $\delta_{R0}$. The changes in steps K and J ($V_K$ and $V_J$) showed that the oxygen-evolving complex and the donor side of PSII were destroyed due to water stress, and that the electron donation capacity to the PSII donor side decreased as a consequence of significantly increased amount of closed PSII RCs (Lazar 2006), especially in maize under EC. However, the C750×D plants significantly increased values of ABS/RC, $TR_0/RC$, and $ET_0/RC$ than other three treatments. Thus, the enhanced energy fluxes (absorbed photon flux, trapping flux, and electron transport flux) per active PSII RC counteracted the downregulation of $\varphi_{Pav}$ and RC/CS in the C750×D plants, and therefore, it improved significantly *J* in Calvin-Benson cycle. While, the $\varphi_{P0}$, *J*,

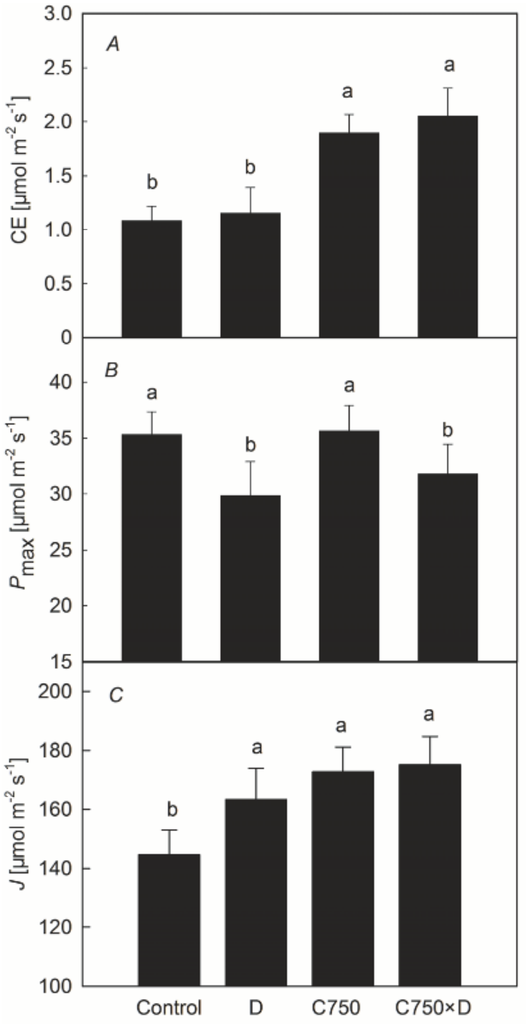

Fig. 4. Performances of carbon reaction under different treatments. *A*: the carboxylation rate (CE); *B*: maximum rate of RuBP regeneration ($P_{max}$); *C*: electron transport rate (*J*). The values represent the means of 3 replications and the bars stand for SE. *Different letters* indicate statistical difference significance at $P<0.05$ among the treatments by *Duncan*'s multiple range test.

Table 2. Relative variable Chl fluorescence at J, I, and K level ($V_J$, $V_I$, and $V_K$). The values are the mean and SE of 8 plants. *Different letters* indicate statistical difference significance at $P<0.05$ among the treatments by *Duncan*'s multiple range test.

| Treatment | $V_J$ | $V_I$ | $V_K$ |
|---|---|---|---|
| Control | $0.246 \pm 0.036^b$ | $0.568 \pm 0.06^b$ | $0.060 \pm 0.023^c$ |
| D | $0.305 \pm 0.052^a$ | $0.643 \pm 0.081^a$ | $0.084 \pm 0.034^b$ |
| C750 | $0.238 \pm 0.034^b$ | $0.606 \pm 0.055^b$ | $0.066 \pm 0.021^c$ |
| C750×D | $0.330 \pm 0.063^a$ | $0.634 \pm 0.098^{ab}$ | $0.123 \pm 0.042^a$ |

$\varphi_{D0}$, and $PI_{total}$ in C(750)×D plants did not significantly increase.

Previous study reported that photosynthetic acclimation of water-stressed plants were often accompanied with the decrease in *J*, which increased the demand of energy dissipation in PSII ($\varphi_{D0}$) (Wilhelm and Selmar 2011). The interactive effects improved PSII performance even more than expected from additive effects (Albert *et al.* 2011). C750×D maintained *J* and CE at the same level as the C750 plants, while high CE could not help C750×D maintain a high $P_{max}$. We suggest that this might be caused by energy oversupply in carbon reaction of the C750×D plants. The increased PSII RC capacity contributed greatly to the enhancement of energy supply. Thus, drought did not cancel out the effects of EC in *J* and CE. Our visual observations showed that a large portion of maize leaves were wilting under water stress at AC, but they remained turgid under water stress at EC.

In summary, by measuring the Chl *a* fluorescence transients and $P_N$-$C_i$ curves, we described the photosynthetic capacity of maize seedlings under interaction of EC and water stress. Compared with water-stressed maize under AC, EC, and water stress reduced RCs and maintained $\varphi_{D0}$ and $\delta_{R0}$ in PSII, but induced more energy to flow into the carbon reaction (*J*) by enhancing the electron transport capacity per RCs. However, the high *J* and CE did not help to increase $P_{max}$ in the water-stressed maize in response to EC.

## References

Ainsworth, E.A., Rogers, A.: The response of photosynthesis and stomatal conductance to rising [CO$_2$]: mechanisms and environmental interactions. − Plant Cell Environ. **30**: 258-270, 2007.

Albert, K.R., Mikkelsen, T.N., Michelsen, A., Ro-Poulsen, H., van der Linden, L.: Interactive effects of drought, elevated CO$_2$ and warming on photosynthetic capacity and photosystem performance in temperate heath plants. − J. Plant Physiol. **168**: 1550-1561, 2011.

de Graaff, M.A., van Groenigen, K.J., Six, J. *et al.*: Interactions between plant growth and soil nutrient cycling under elevated CO$_2$: a meta-analysis. − Global Change Biol. **12**: 2077-2091, 2006.

Furbank, R.T., Chitty, J.A., von Caemmerer, S., Jenkins, C.L.D.: Antisense RNA inhibition of RbcS gene expression reduces Rubisco level and photosynthesis in the C$_4$ plant *Flaveria bidentis*. − Plant Physiol. **111**: 725-734, 1996.

Ghannoum, O., Evans, J.R., von Caemmerer, S.: Nitrogen and water use efficiency in C$_4$ plants. – In: Raghavendra, A.S., Sage, R.F. (ed.): C$_4$ photosynthesis and related CO$_2$ concentrating mechanisms Pp. 129-146. Springer, Dordrecht 2011.

Ghannoum, O., von Caemmerer, S., Ziska, L.H., Conroy, J.P.: The growth response of C$_4$ plants to rising atmospheric CO$_2$ partial pressure: a reassessment. − Plant Cell Environ. **23**: 931-942, 2000.

Grodzinski, B., Jiao, J., Leonardos, E.D.: Estimating photosynthesis and concurrent export rates in C$_3$ and C$_4$ species at ambient and elevated CO$_2$. − Plant Physiol. **117**: 207-215, 1998.

Guan, X., Gu, S.: Photorespiration and photoprotection of grapevine (*Vitis vinifera* L. cv. Cabernet Sauvignon) under water stress. − Photosynthetica **47**: 437-444, 2009.

Gutiérrez, D., Gutiérrez, E., Pérez, P. *et al.*: Acclimation to future atmospheric $CO_2$ levels increases photochemical efficiency and mitigates photochemistry inhibition by warm temperatures in wheat under field chambers. − Physiol. Plantarum **137**: 86-100, 2009.

Jin, S.-H., Wang, D., Zhu, F.-Y. *et al.*: Up-regulation of cyclic electron flow and down-regulation of linear electron flow in antisense-*rca* mutant rice. − Photosynthetica **46**: 506-510, 2008.

Lawlor, D.W., Tezara, W.: Causes of decreased photosynthetic rate and metabolic capacity in water-deficient leaf cells: a critical evaluation of mechanisms and integration of processes. − Ann. Bot. **103**: 561-579, 2009.

Lazár, D.: Modelling of light-induced chlorophyll *a* fluorescence rise (O-J-I-P transient) and changes in 820 nm-transmittance signal of photosynthesis. − Photosynthetica **47**: 483-498, 2009.

Lazár, D., Nauš, J., Matoušková, M., Flašarová, M.: Mathematical modeling of changes in chlorophyll fluorescence induction caused by herbicides. − Pestic. Biochem. Phys. **57**: 200-210, 1997.

Lazár, D.: The polyphasic chlorophyll *a* fluorescence rise measured under high intensity of exciting light. − Funct. Plant Biol. **33**: 9-30, 2006.

Long, S.P., Ainsworth E.A., Rogers A., Ort D.R.: Rising atmospheric carbon dioxide: Plants FACE the future. − Annu. Rev. Plant Biol. **55**: 591-628, 2004.

Markelz, R.J.C., Strellner, R.S., Leakey, A.D.B.: Impairment of $C_4$ photosynthesis by drought is exacerbated by limiting nitrogen and ameliorated by elevated [$CO_2$] in maize. − J. Exp. Bot. **62**: 3235-3246, 2011.

Maroco, J.P., Edwards, G.E., Ku, M.S.B.: Photosynthetic acclimation of maize to growth under elevated levels of carbon dioxide. − Planta **210**: 115-125, 1999.

Martínez-Carrasco, R., Pérez, P., Morcuende, R.: Interactive effects of elevated $CO_2$, temperature and nitrogen on photosynthesis of wheat grown under temperature gradient tunnels. − Environ. Exp. Bot. **54**: 49-59, 2005.

Oukarroum, A., Schansker, G., Strasser, R.J.: Drought stress effects on photosystem I content and photosystem II thermotolerance analyzed using Chl *a* fluorescence kinetics in barley varieties differing in their drought tolerance. − Physiol. Plantarum **137**: 188-199, 2009.

Prins, A., Mukubi, J.M., Pellny, T.K. *et al.*: Acclimation to high $CO_2$ in maize is related to water status and dependent on leaf rank. − Plant Cell Environ. **34**: 314-331, 2011.

Reich, P.B., Hungate, B.A., Luo, Y.Q.: Carbon-nitrogen interactions in terrestrial ecosystems in response to rising atmospheric carbon dioxide. − Annu. Rev. Ecol. Evol. S. **37**: 611-636, 2006.

Sage, R.F., Kubien, D.S.: Quo vadis $C_4$? An ecophysiological perspective on global change and the future of $C_4$ plants. − Photosynth. Res. **77**: 209-225, 2003.

Sarker, B.C., Hara, M.: Effects of elevated $CO_2$ and water stress on the adaptation of stomata and gas exchange in leaves of eggplants (*Solanum melongena* L.). − Bangl. J. Bot. **40**: 1-8, 2011.

Schansker, G., Tóth, S.Z., Strasser, R.J.: Methylviologen and dibromothymoquinone treatments of pea leaves reveal the role of photosystem I in the Chl a fluorescence rise OJIP. – Biochim. Biophys. Acta-Bioenergetics **1706**: 250-261, 2005.

Srivastava, A., Guisse, B., Greppin, H., Strasser, R.J.: Regulation of antenna structure and electron transport in Photosystem II of *Pisum sativum* under elevated temperature probed by the fast polyphasic chlorophyll *a* fluorescence transient: OKJIP. – Biochim. Biophys. Acta-Bioenergetics **1320**: 95-106, 1997.

Stirbet, A., Govindjee, Strasser, B.J., Strasser, R.J.: Chlorophyll *a* fluorescence Induction in higher plants: Modelling and numerical simulation. − J. Theor. Biol. **193**: 131-151, 1998.

Stitt, M.: Rising $CO_2$ levels and their potential significance for carbon flow in photosynthetic cells. − Plant Cell Environ. **14**: 741-762, 1991.

Strasser, B.J., Strasser, R.J. Measuring fast fluorescence transients to address environmental questions: the JIP test. − In: Mathis, P. (ed.): Photosynthesis: From Light to Biosphere. Pp. 997-980, Kluwer Academic, Dordrecht 1995.

Strasser, R., Tsimilli-Michael, M., Srivastava, A.: Analysis of the chlorophyll *a* fluorescence transient. − In: Papageorgiou, G.C., Govindjee (ed.): Chlorophyll *a* Fluorescence: A Signature of Photosynthesis. Pp. 321-362. Springer, Dordrecht 2004.

Strasser, R.J., Tsimilli-Michael M., Qiang, S., Goltsev, V.: Simultaneous *in vivo* recording of prompt and delayed fluorescence and 820-nm reflection changes during drying and after rehydration of the resurrection plant *Haberlea rhodopensis*. – Biochim. Biophys. Acta-Bioenergetics **1797**: 1313-1326, 2010.

Taiz, L., Zeiger, E.: Photosynthesis: the light reactions − In: Taiz, L., Zeiger, E. (ed.): Plant Physiology. Pp. 70-71; 124-135, Sinauer Associates, Inc., Sunderland 2006.

Tsimilli-Michael, M., Strasser, R.J.: *In vivo* assessment of stress impact on plant's vitality: applications in detecting and evaluating the beneficial role of mycorrhization on host plants. − In: Varma, A. (ed.): Mycorrhiza: Genetics and Molecular Biology, Eco-function, Biotechnology, Ecophysiology, and Structure and Systematics. Pp. 679-703. Springer, Berlin 2008.

von Caemmerer, S., Furbank, R.: Modeling $C_4$ photosynthesis. − In: Sage, R.F., Monson, R.K. (ed.): $C_4$ Plant Biology. Pp. 173-211, Academic Press, San Diego 1999.

Vu, J.C.V., Allen, L.H.: Growth at elevated $CO_2$ delays the adverse effects of drought stress on leaf photosynthesis of the $C_4$ sugarcane. − J. Plant Physiol. **166**: 107-116, 2009.

Wand, S.J.E., Midgley, G.F., Jones, M.H., Curtis, P.S.: Responses of wild $C_4$ and $C_3$ grass (Poaceae) species to elevated atmospheric $CO_2$ concentration: a meta-analytic test of current theories and perceptions. − Global Change Biol. **5**: 723-741, 1999.

Wilhelm, C., Selmar, D.: Energy dissipation is an essential mechanism to sustain the viability of plants: The physiological limits of improved photosynthesis. − J. Plant Physiol. **168**: 79-87, 2011.

Zhou, Z.C., Shangguan, Z.P.: Effects of elevated $CO_2$ concentration on the biomasses and nitrogen concentrations in the organs of sainfoin (*Onobrychis viciaefolia* Scop.). − Agr. Sci. China. **8**: 424-430, 2009.

Zhu, X.-G., Govindjee, Baker, N.R. *et al.*: Chlorophyll a fluorescence induction kinetics in leaves predicted from a model describing each discrete step of excitation energy and electron transfer associated with Photosystem II. − Planta **223**: 114-133, 2005.