

THE EFFECTS OF CD²⁺ STRESS ON PHOTOSYSTEM II FUNCTIONING OF RICE (*ORYZA SATIVA* L.) LEAVES UNDER ELEVATED CO₂ LEVEL

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Abstract. The effects of elevated levels of CO₂ (EC) and cadmium (Cd²⁺) on the photosystem II of rice leaves were studied using fast chlorophyll fluorescence technique. Rice seedlings (two-leaves-stage) were treated under atmospheric CO₂ (AC, 400 µmol/mol) and elevated CO₂ (EC, 800 µmol/mol), while applied 0, 50, 150, 250 and 500 µmol/L Cd²⁺ concentrations respectively. Chlorophyll fluorescence parameters were measured after six days treatments. The results showed that: (1) Under Cd²⁺, F_V/F₀, φPo, Ψo, φEo and PI_{ABS} decreased significantly, while V_K, V_J, W_K, M₀, φDo, ABS/RC, TR₀/RC, DI₀/RC increased significantly compared to AC. Additional effects were found on the donor side, reaction centers and acceptor side under Cd²⁺, which caused a lower energy connectivity, reduction of PS II activity and electron transfer efficiency as well as an increase of heat dissipation. (2) EC significantly increased F_V/F₀, φPo, Ψo, φEo and PI_{ABS}, while significantly decreased V_J and φDo compared to AC. EC increased the photo-energy conversion efficiency of PS II. (3) Under EC and Cd²⁺ treatments, the adverse effects of Cd²⁺ were slightly alleviated by EC in EC 50 (EC + 50 µmol/L Cd²⁺, the following abbreviations are consistent with this), but the negative influence on photosystem II was still the most significant.

Keywords: *JIP-Test, OJIP curve, quantum efficiency, specific energy fluxes, performance index*

Introduction

Most of the heavy metal pollutions in soil are from the industrial wastewater discharge, agricultural phosphate fertilizer and sewage sludge, and have seriously affected much farmland and crops (Zou et al., 2020). Cadmium and mercury pollutions are especially severer than other elements (Xiao et al., 2019; Hang et al., 2009). The plants under Cd²⁺ stress will affect in cell damages, production of toxic metabolites, inhibition of photosynthesis and respiration (Hassan et al., 2014; Meng et al., 2009; Liu et al., 2012; Singh et al., 2016). Recently, the NOAA (national oceanic and atmospheric administration, USA) detected CO₂ concentrations in the atmosphere as high as 414.7 µmol/mol peak, and it will continue to grow in the coming years (Kalva et al., 2014; Killi et al., 2018; Frew and Prince, 2019). Photosynthesis, as a very important metabolic reaction in green plants, responds to heavy metal stresses very quickly (Belatik et al., 2013). Fast chlorophyll fluorescence can reflect the state of photosystem II (PSII), the main part of light reaction in photosynthesis, and it has been widely used because of its convenience and non-destructiveness (Strasser et al., 2004; Chen et al., 2016). In this study, the rapid chlorophyll fluorescence induction curves and parameters of rice (*Oryza sativa* L.) seedling leaves under Cd²⁺ and/or EC treatments were tested, so as to explore the photosynthetic effects of plants under the environmental changes.

Materials and methods

Rice seedlings (Beijing 2, which has been widely planted in Liaoning province, China) were cultured under Hoagland nutrient solution with the control of 26 °C/22 °C day/night, 16 h/8 h light/dark period, 3000 lux illumination intensities using carbon dioxide artificial climate box. When the seedlings grew to the two-leaves-stage, applied ten treatments, each treatment had 6 pots (*Table 1*).

Table 1. Name of treatments

Cd ²⁺ (μmol/L)	0	50	150	250	500
AC (400 μmol/mol CO ₂)	AC	AC 50	AC 150	AC 250	AC 500
EC (800 μmol/mol CO ₂)	EC	EC 50	EC 150	EC 250	EC 500

After 6 days treatments, leaves of rice seedlings were measured by Pocket PEA (Hanshatech, UK) during 11:00-12:00 which had 20 min dark adaption before measurements. The positions of measurements were located in the middle and front of the second complete leaves. Three repeats were selected randomly in each pot (6 pots/treatment, a total of 18 repeats/treatment).

According to the Kautsky effect, the fluorescence change process from O step to P step is the fast chlorophyll fluorescence induction kinetic curve, and the data analysis and processing for this curve is summarized as JIP-test (Strasser et al., 2004; Guisse et al., 1995). The parameters of JIP-test are presented in *Table 2*. According to the JIP-test, the fluorescence OJIP transients were analyzed. Variable fluorescence $V_{OP} = (F_t - F_0)/(F_P - F_0)$ (Eq. 1) were normalized on a logarithmic time scale while $V_{OK} = (F_t - F_0)/(F_K - F_0)$ (Eq. 2) and $V_{OJ} = (F_t - F_0)/(F_J - F_0)$ (Eq. 3) were normalized on a linear time scale. And the different kinetics, ΔV_{OK} and ΔV_{OJ} of treatments versus AC, can show normalized transients between the O, J, I, P steps (Li et al., 2014), which were called L-band and K-band respectively.

The significant differences of parameters were using the method of two-way ANOVA followed by LSD's multiple-range test for multiple comparisons. The data analysis was done with the SPSS statistical software (v20.0, SPSS, USA) and the figures were drawn by Origin (v9.0, Origin, USA) software.

Results

OJIP curve

The typical OJIP curve suggested that all leaves were photosynthetically active, but all curves showed inhibition tendency except under EC compared with AC. Fluorescence intensity under EC were the highest at phases I and P steps, and fluorescence intensity under AC 500 was the lowest at J, I and P steps. Fluorescence intensity of Cd²⁺ treatments was lower than AC at I and P steps (*Fig. 1*).

Fluorescence data between O step and P step (V_{OP}) were deviation normalized under different treatments. V_{OP} under Cd²⁺ treatments were higher than AC. However, there was no significant difference between different Cd²⁺ treatments. V_{OP} under EC was lower than AC (*Fig. 2*).

Table 2. Summary of parameters, formulae and their description using JIP-test for the analysis of the fluorescence transient O-J-I-P

Fluorescence parameters	Description
<i>Fluorescence parameters derived from the extracted data</i>	
F_t	Fluorescence intensity at time t after onset of actinic illumination
F_0	Minimal reliable recorded fluorescence, at 50 μ s
F_p	Maximum fluorescence, when all PS II RCs are closed
F_K	Fluorescence intensity at 300 μ s
F_v/F_0	Potential photochemical efficiency
$V_K = (F_K - F_0)/(F_M - F_0)$	Relative variable fluorescence intensity at the K-step
$V_J = (F_J - F_0)/(F_M - F_0)$	Relative variable fluorescence intensity at the J-step
$W_K = (F_K - F_0)/(F_J - F_0)$	The ratio of K step relative variable fluorescence to J step
$M_0 = 4(F_K - F_0)/(F_M - F_0)$	Approximated initial slope of the fluorescence transient
$Sm = (Area)/(F_M - F_0)$	Normalized total complementary area above the O-J-I-P transient
<i>Yields or flux ratios</i>	
$\phi Po = TR_0/ABS = [1 - (F_0/F_M)]$	Maximum quantum yield of primary photochemistry (at t = 0)
$\Psi_0 = ET_0/TR_0 = (1 - V_J)$	Probability (at t = 0) that a trapped exciton moves an Electron into the electron transport chain beyond Q_A^-
$\phi Eo = ET_0/ABS = [1 - (F_0/F_M)] \Psi_0$	Quantum yield of electron transport (at t = 0)
$\phi Do = 1 - \phi Po$	Quantum yield (at t = 0) of energy dissipation
<i>Specific energy fluxes (per Q_A^- reducing PS II reaction center)</i>	
$ABS/RC = M_0(1/V_J)(1/\phi Po)$	Absorption flux per RC
$TR_0/RC = M_0(1/V_J)$	Trapped energy flux per RC (at t = 0)
$ET_0/RC = M_0(1/V_J) \Psi_0$	Electron transport flux per RC (at t = 0)
$DI_0/RC = (ABS/RC) - (TR_0/RC)$	Dissipated energy flux per RC (at t = 0)
<i>Performance index</i>	
$PI_{ABS} = (RC/ABS)[\phi Po/(1 - \phi Po)][\Psi_0/(1 - \Psi_0)]$	Performance index on absorption basis

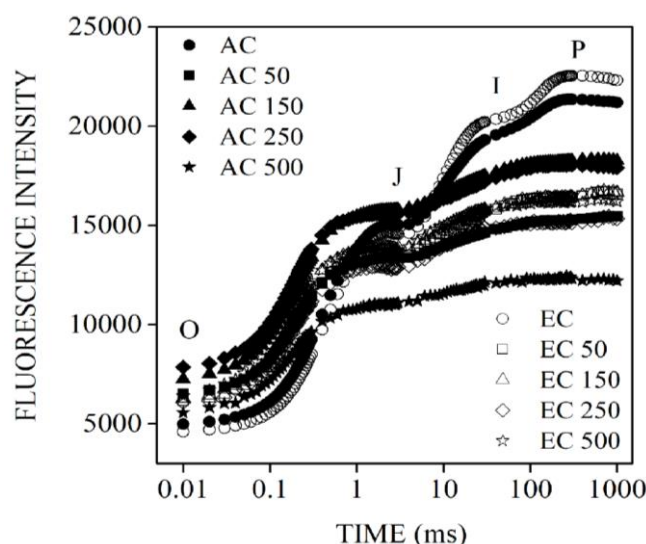


Figure 1. Chl a fluorescence intensity of different treatments (n = 18)

L-band and K-band

The L-band reflects the energy connection of PS II units, and the negative L-band indicates a high system connection degree or high excitation energy utilization rate. In

our study, V_{OK} and ΔV_{OK} showed the energy connection of PS II was higher under EC. Cd²⁺ treatments result in a destruction of PS II energy connectivity, and the destruction became severer with the increases of Cd²⁺ concentration (Fig. 3A).

The K-band reflects the activity of the oxygen evolving complex (OEC). A negative K-band indicates the intactness of the functional antenna. Similar to the L-band, only EC presented a negative band, while seedlings under Cd²⁺ stress all showed OEC damage in different degrees (Fig. 3B).

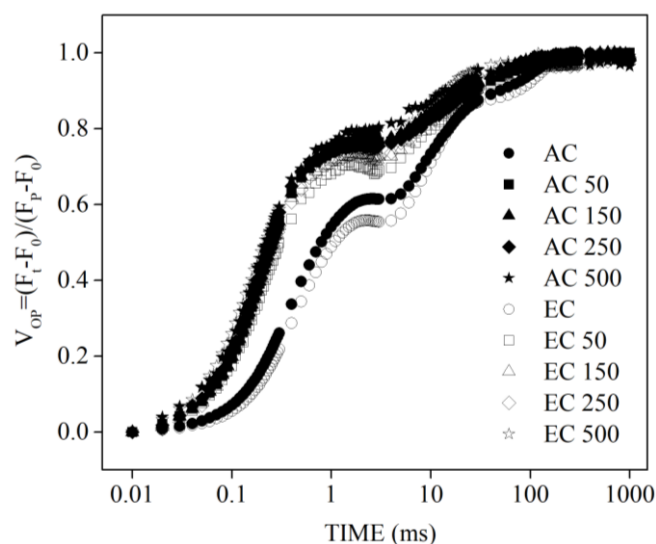


Figure 2. V_{OP} on the logarithmic scale ($n = 18$)

Kinetic parameters changes of rapid chlorophyll fluorescence induction

Under Cd²⁺ treatments, F_V/F_0 were significantly lower, while V_K , V_J , W_K , M_0 were significantly higher compared with AC ($P < 0.01$). Under EC, F_V/F_0 was significantly higher, while V_J was significantly lower than AC ($p < 0.05$). Under combined treatments, V_K , V_J and M_0 under EC 50 treatment were significantly lower than AC 50 ($p < 0.05$). Sm was significantly higher under EC 150 and EC 250 compared with the same concentrations under AC ($p < 0.05$). Under EC 500, F_V/F_0 and Sm were significantly higher than AC 500 ($p < 0.05$) (Fig. 4).

ϕPo , Ψ_o and ϕEo were significantly lower, but ϕDo was significantly higher under Cd²⁺ treatments compared with AC ($p < 0.01$). Under EC, ϕPo , Ψ_o , and ϕEo were significantly higher, but ϕDo were significantly lower compared with AC ($p < 0.05$). Under EC 50, ϕEo and Ψ_o were significantly higher than AC 50 ($p < 0.01$). Under EC 500, ϕPo and ϕEo were significantly higher but ϕDo was significantly lower compared with AC 500 ($p < 0.05$) (Fig. 5).

Under Cd²⁺ treatments, ABS/RC , TR_o/RC and DI_o/RC were all significantly higher compared with AC ($P < 0.01$). Under combined treatments, ET_o/RC was significantly higher in EC 50 compared with AC 50 ($p < 0.05$). Under EC 500, ET_o/RC was significantly higher, but DI_o/RC was significantly lower than AC 500 ($p < 0.05$) (Fig. 6).

PI_{ABS} was significantly decreased compared with AC ($p < 0.05$). Under EC, PI_{ABS} was significantly higher than AC ($p < 0.05$). Under the combined treatments, PI_{ABS} of EC 50 was significantly higher than AC 50 ($p < 0.05$) (Fig. 7).

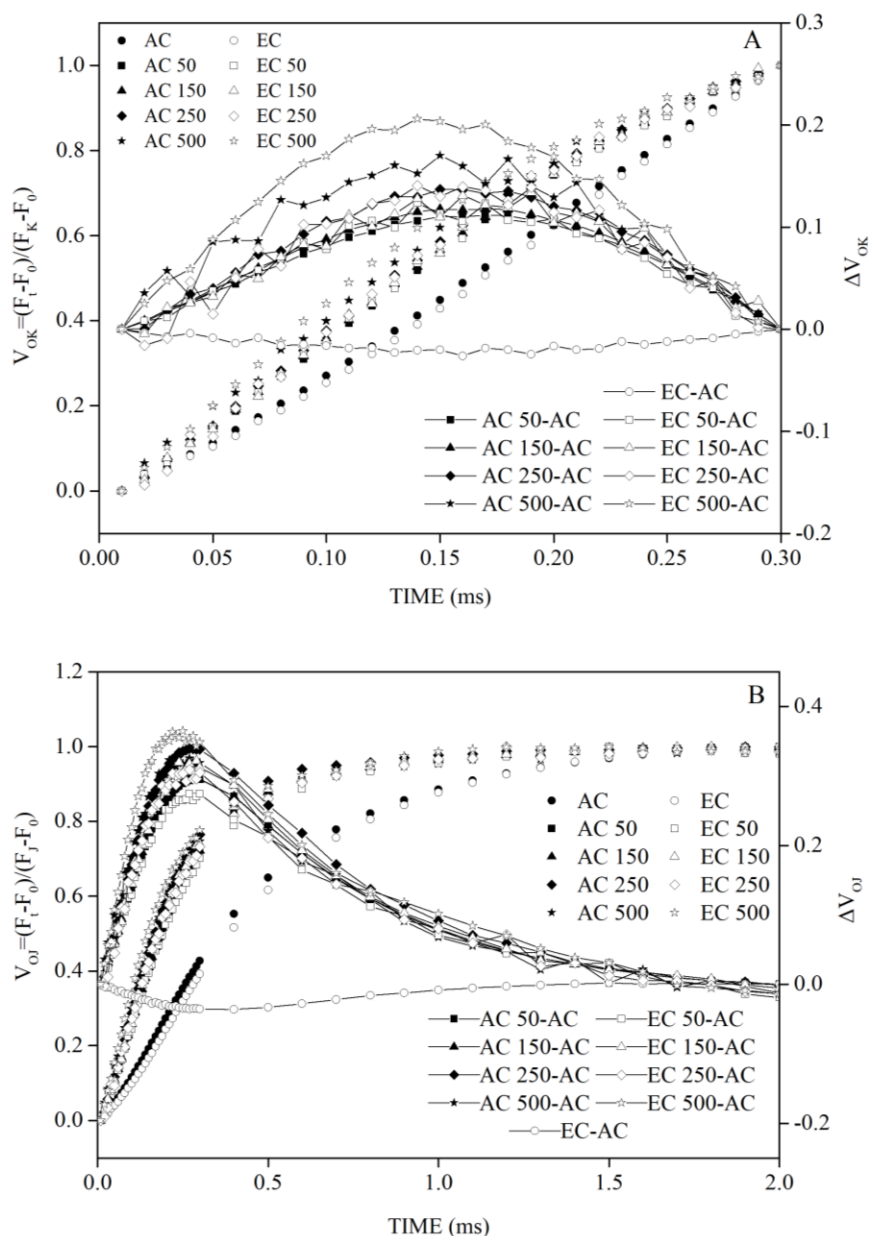


Figure 3. (A) V_{OK} and ΔV_{OK} ; (B) V_{OJ} and ΔV_{OJ} . In (A) and (B), open symbol curves represent left y-axis and closed symbol curves represent right y-axis. Left axis shows the V_{OK} and V_{OJ} . Right axis shows the ΔV_{OK} and ΔV_{OJ} stand for stress treatments versus control (AC). ΔV_{OK} and ΔV_{OJ} revealing the L-band and K-band respectively ($n = 18$)

Discussion

The effects of Cd²⁺ stress

Our study showed that Cd²⁺ stress improved the K step of the OJIP curve, and also caused positive K-band and L-band. W_K is the ratio of variable fluorescence F_K to the amplitude $F_J - F_0$, and V_K is the relative variable fluorescence intensity at the K step. Both these two parameters reflect the damage of OEC. In our results, Cd²⁺ treatment caused lower energy connectivity and destruction of OEC intactness. The electron transfer on the PS II donor side was also affected.

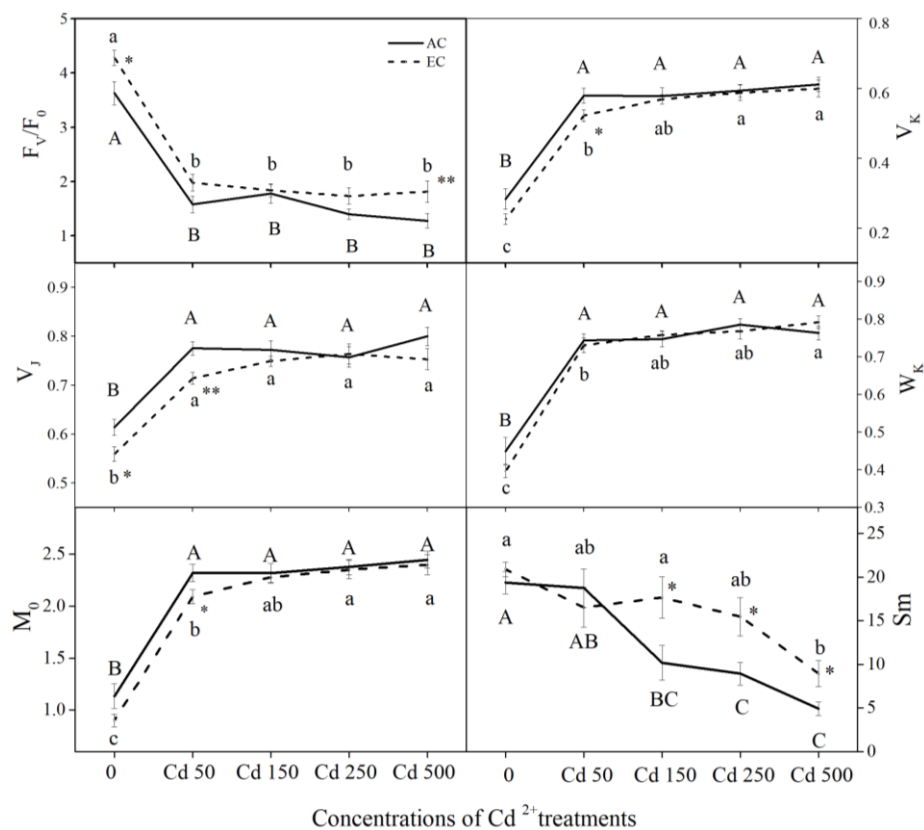


Figure 4. The variation trend and significant difference of fluorescence parameters under different treatments. The bars indicated standard error. For the same CO₂ treatments, significance differences were marked as ABC (for AC) and abc (for EC) at $P < 0.05$ (LSD test). For the same Cd²⁺ concentration, an asterisk was marked to indicates significant difference in comparison with AC (* $P < 0.05$; ** $P < 0.01$). The following figures are consistent with this figure ($n = 18$)

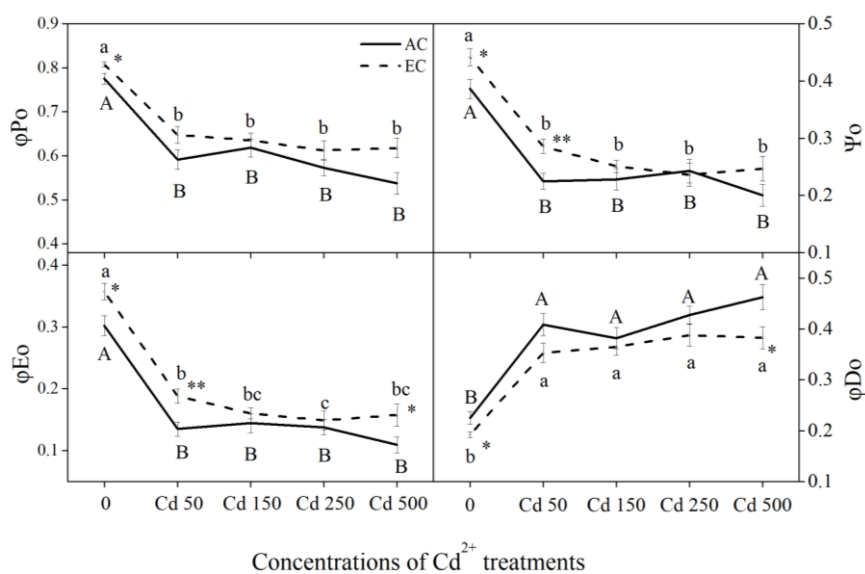


Figure 5. The variation trend and significant difference of quantum efficiency under different treatments ($n = 18$)

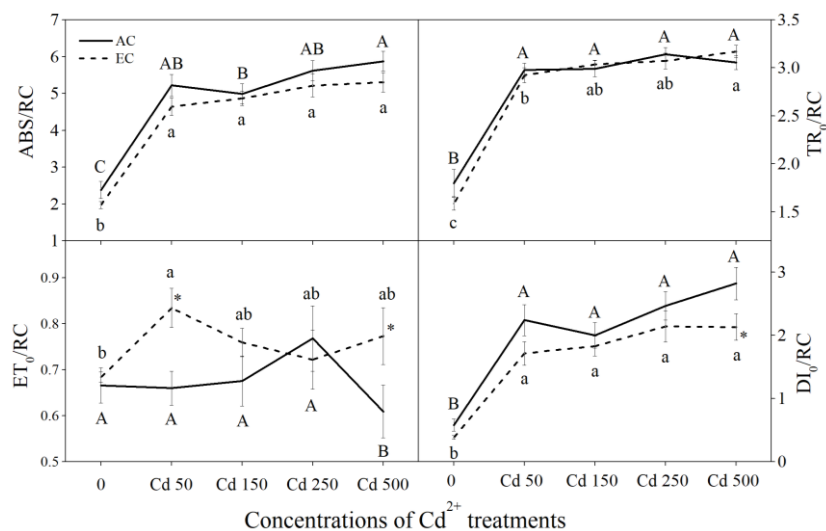


Figure 6. The variation trend and significant difference of specific energy fluxes under different treatments ($n = 18$)

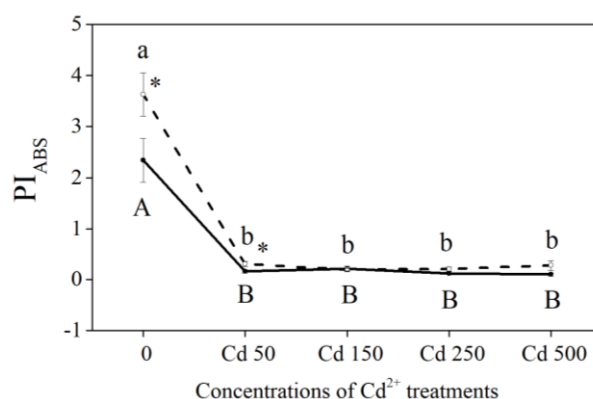


Figure 7. The variation trend and significant difference of comprehensive performance index under different treatments ($n = 18$)

M₀ is the initial slope of the OJIP curve, reflecting the rate at which Q_A's reduction during O-J. Sm is the integral area of normalized OJIP curve, reflecting the energy required when Q_A is completely reduced, that is, reflecting the capacity of plastoquinone (PQ) (Strasser et al., 1995) on the acceptor side of PS II reaction center. Ψ₀ reflects the ability of PS II to transfer electrons to the downstream electron transport chain. Our study showed that in PS II acceptor side, decreases in Sm and Ψ₀ and the increase in M₀ compared with AC showed that the activity of the electron transport beyond Q_A was inhibited under Cd²⁺ stress. This is the same as Chu's results (Chu et al., 2018). φE₀ reflects the quantum yield for electron transport in the reaction center. φD₀ represents the maximum quantum yield of non-photochemical de-excitation (Li and Zhang, 2015). In this study, the capacity of PQ pool on the acceptor side decreased with the increase of Cd²⁺ concentrations, which led to the increase of φE₀ and φD₀, that is, the quantum ratio of PS II reaction center for heat dissipation increased under Cd²⁺ stress, and the quantum ratio for electron transfer decreased. The Q_A reduction

accumulates, and more surplus energy was used to reduce Q_A, which hinders the electron transfer. The ability of PS II to transfer electrons to the downstream electron transport chain was destroyed by Cd²⁺. The electron transfer on the acceptor side was inhibited.

F_v/F₀ reflects the potential activity of PS II, which is proportional to the amount of PS II RCs (Luo et al., 2016) and is often used to judge whether the leaves of plants are photo-inhibition. In our study, Cd²⁺ treatment significantly reduced F_v/F₀ compared with AC. This is similar to the result of Essemine et al. (2020). φPo reflects the maximum photochemical quantum efficiency of the PS II reaction center which is generally stable and rarely affected by growth conditions (Kramer et al., 2004), but decreases under the stress condition (Baker, 2008). It can be seen from our results that F_v/F₀ and φPo had the same downward trends under Cd²⁺ stress, which might be the PS II reaction center had photo-inhibition and PS II electron transfer was blocked. V_J represents the degree of closure of the reactive center of PS II. Under the treatments of Cd²⁺, the number of RCs decreased, and a large number of RCs closed.

Specific energy flux includes ABS/RC, TR₀/RC, ET₀/RC, DI₀/RC etc. (Strasser et al., 2004), which reflecting the activity of PS II reaction center when Q_A is in reducible state. Our results showed that under Cd²⁺ treatments, the absorption flux per RC (ABS/RC), the energy flux trapped per RC (TR₀/RC) and the dissipated energy fluxes per RC (DI₀/RC) all significantly higher than AC, while the electron transport flux per RC (ET₀/RC) was significantly lower under AC 500. Increase in trapping per RC (TR₀/RC) can indicate impairment of the oxygen evolving complex (Kalaji et al., 2014; Franić et al., 2018). After absorbed by PS II reaction center, the light was trapped by reaction center (TR₀/RC), the ABS/RC was mainly used for electron transport flux ET₀/RC and dissipated flux DI₀/RC (Strasser et al., 2010; Tsimilli-Michael and Strasser, 2008). It can be found from our results that, although the light energy absorbed and trapped by unit RC increased under the stress of Cd²⁺, the light energy used for electron transfer increased limited, so more light energy consumption occurred in dissipation flux, while photosynthesis did not increase much. This is the same as Xue's results (Xue et al., 2018). A study suggested that plants dissipate excess light energy which was not available for photosynthesis in order to reduce damage to PS II reactive centers (Appenroth et al., 2001). In our study, rice leaves may also had such a protective mechanism.

PI_{ABS}, a comprehensive performance index based on light absorption (Brestic et al., 2012; Heerden et al., 2004) which reflects the overall function of PS II system, is the most sensitive parameter to the stressed environment. It was significantly reduced by Cd²⁺ and inversely proportional to Cd²⁺ concentration in our study. The result showed that Cd²⁺ had obvious damage to photosynthetic performance of rice seedlings.

The effects of EC

Elevated CO₂ has been proved to be able to enhanced photosynthesis of C₃ plants (Lahijani et al., 2018). The OJIP curve under EC was higher at I and P steps than AC, which means the promotion of electron transport at the donor side of PS II. The change in fluorescence is the result of variation in the redox state of the PS II RCs complex (Haldimann and Strasser, 1999). Strasser et al. (1995) reported that the decrease of electron transport beyond Q_A⁻ results the high fluorescence. In our study, the difference

kinetics ΔV_{OP} revealed that seedlings under EC produced lower fluorescence, which showed less biochemical inhibition. EC produced a negative K-band and L-band, suggesting better energetic connectivity of PS II and photosynthetic performance compared with AC.

Under EC, M_0 and S_m significantly decreased compared with AC, that is, the degree of openness of the RCs increased and the proportion of the inactivated RCs decreased, which was conducive to the electron transfer down from Q_A^- . The increases in Ψ_o and ϕE_o under EC indicated that EC promoted the primary light reaction and the redox reactions after Q_A .

Both F_v/F_0 and ϕP_o under EC showed an increasing trend in different degrees, indicating that the increase of CO₂ concentration enhanced the electron transfer efficiency and the PS II light energy conversion. Compared with AC, ϕD_o and V_J were significantly reduced by EC, indicating that EC reduced the heat dissipation and increases the opening degree of RCs. In conclusion, photo-energy conversion efficiency of PS II active center can be alleviated under elevated CO₂.

In the composite index of PI_{ABS} , EC significantly improved it compared with AC. The result showed that EC could improve the photosynthetic performance of rice seedlings.

The effects of Cd²⁺ stress and EC

In our study, the K-band and L-band showed that the alleviated effect of EC on PS II donor side was not obvious under Cd²⁺ stress. Under EC 50, the significant decrease of V_K compared with AC 50 indicated that EC could alleviate the damage of OEC caused by lower Cd²⁺ stress.

Compared with AC 50, EC 50 had a significant decrease in M_0 and a significant increase in Ψ_o and ϕE_o . These suggested that reduction of Q_A to Q_A^- was higher in EC 50 than AC 50. There were no significant differences in Ψ_o and ϕE_o under EC 150, EC 250 and EC 500 compared with the same Cd²⁺ concentrations under AC. To sum up, elevated CO₂ alleviates lower concentration Cd²⁺ stress to a certain extent in terms of PS II energy distribution.

In the specific fluxes, ET_0/RC was significantly higher under EC 50 compared with AC 50. There were no significant differences in ABS/RC and TR_0/RC under EC 50, EC 150, EC 250 and EC 500 compared with the same Cd²⁺ concentrations under AC. V_J was significantly decreased under EC 50 compared with AC 50. It can be seen that the degree of closure of PS II active RCs was alleviated, and the electron transport was significantly increased in lower Cd²⁺ treatment under EC. Under some stresses, PS II RCs were reversibly inactivated and became an energy trap, which could absorb light energy but could not promote electron transfer (Lee et al., 2001). In our study, EC can alleviate the energy trap, but the effect of cadmium stress cannot be completely eliminated.

PI_{ABS} of EC 50 was significantly higher than AC 50. In terms of the comprehensive performance index, the effect of EC had some mitigation on 50 $\mu\text{mol/L}$ Cd²⁺, but was not particularly obvious in other concentrations.

Compared with the same Cd²⁺ concentrations under AC treatments, EC 150, EC 250 and EC 500 showed little alleviated effects on specific energy flux, quantum efficiency and comprehensive performance index.

Conclusions

In this study, the effects of elevated CO₂ and/or Cd²⁺ stress in leaves of rice were studied using the rapid chlorophyll fluorescence technique. The main results were as follows: (1) Cd²⁺ stress damaged the integrity of OEC in rice seedling leaves, which result in lower energy connectivity at the PS II donor side. Also, the effects on receptor side and the reaction centers were the decrease of RCs' number and the inhibition of the electron transfer. RCs absorbed more energy but most of the energy was used for heat dissipation; (2) Short-term elevated CO₂ can promote electron transfer in PS II donor side of rice seedling leaves. The photo-energy conversion efficiency of PS II increased with the increase of the openness of the RC; (3) Short-term elevated CO₂ can relieve the adverse effects of lower concentration Cd²⁺ treatment, but it cannot significantly eliminate the damage of higher Cd²⁺ stress. (4) In the future study, the intrinsic molecular mechanism changes of chlorophyll fluorescence should be concerned.

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