BIOLOGIA PLANTARUM 52 (2): 307-313, 2008

Water-water cycle involved in dissipation of excess photon energy in phosphorus deficient rice leaves

X.-Y. WENG*, H.-X. XU, Y. YANG and H.-H. PENG

National Laboratory of Plant Physiology and Biochemistry, Department of Biological Science, College of Life Science, Zhejiang University, Hangzhou, Zhejiang, 310058, China

Abstract

The water-water cycle which may be helpful for dissipating the excitation pressure over electron transport chain and minimizing the risk of photoinhibition and photodamage was investigated in rice after 10-d P-deficient treatment. Net photosynthetic rate decreased under P-deficiency, thus the absorption of photon energy exceeded the energy required for CO_2 assimilation. A more sensitive response of effective quantum yield of photosystem 2 (Φ_{PS2}) to O_2 concentration was observed in plants that suffered P starvation, indicating that more electrons were transported to O_2 in the P-deficient leaves. The electron transport rate through photosystem 2 (PS 2) (J_f) was stable, and the fraction of electron transport rate required to sustain CO_2 assimilation and photorespiration (J_g/J_f) was significantly decreased accompanied by an increase in the alternative electron transport (J_a/J_f), indicating that a considerable electron amount had been transported to O_2 during the water-water cycle in the P-deficient leaves. However, the fraction of electron transport to photorespiration (J_o/J_f) was also increased in the P-deficient leaves and it was less sensitive than that of water-water cycle. Therefore, water-water cycle could serve as an efficient electron sink. The higher non-photochemical fluorescence quenching (q_N) in the P-deficient leaves depended on O_2 concentration, suggesting that the water-water cycle might also contribute to non-radiative energy dissipation. Hence, the enhanced activity of the water-water cycle is important for protecting photosynthetic apparatus under P-deficiency in rice.

Additional key words: *Oryza sativa*, net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, photosystem 2, chlorophyll *a* fluorescence, non-photochemical and photochemical quenching, photorespiration.

Introduction

Phosphorus (P) is one of the most important elements involved in plant growth and metabolism. P-deficiency significantly depresses CO_2 assimilation capacity (Rao and Terry 1989, Jacob and Lawlor 1991, 1993, Qiu and Israel 1994, Maleszewski *et al*. 2004). CO_2 assimilation is the major consumer for photon energy absorbed in antenna pigments and decrease in photosynthetic rate will inevitably result in accumulation of excess photon energy under high irradiance. If not dissipated safely, the excess energy will cause damages of photosynthetic apparatus (Demmig-Adams and Adams 1992). Hence, safe dissipation of excess photons and electrons is especially critical for protecting the photosynthetic apparatus from damage under P-deficiency. Water-water cycle is one of multiple photoprotective mechanisms within the chloroplast developed in plants to cope with the potentially damaging effects of excess energy (Niyogi 1999, 2000).

Since Mehler (1951) observed that chloroplasts could use oxygen as an electron acceptor in a Hill reaction, Mehler peroxide reaction (water-water cycle) has often been studied as a mechanism with possible function in the photoprotection of plants (Asada 1999). In tropical trees, the linear electron flow through the water-water cycle

Received 23 January 2006, a*ccepted* 25 October 2006.

Abbreviations: APX - ascorbate peroxidase; c_i - intercellular CO_2 concentration; Chl - chlorophyll; g_s - stomatal conductance; FM - fresh mass; J_a - the rate of alternative electron transport; J_f - the electron transport rate through PS2; J_g - the rate of electron transport required to maintain photosynthetic carbon reduction cycle (PCR) and photorespiratory carbon oxidation cycle (PCO); J_o - the rate of electron transport though photorespiration; MDA - malonyldialdehyde; $O_2^{\bullet -}$ - superoxide radical; P_N - net photosynthetic rate; PCO - photorespiratory carbon oxidation cycle; PCR - photosynthetic carbon reduction cycle; PPFD - photosynthetic photon flux density; Pr - protein; PS 2 - photosystem 2; q_P - photochemical quenching; q_N - non-photochemical quenching; SOD - superoxide dismutase; TBA - barbiturate; TCA, - trichloroacetic acid; Φ_{PS2} - effective PS2 quantum yield.

Acknowledgements: This work was supported by Natural Science Foundation of Zhejiang Province in China (M303032).

* Corresponding author; e-mail: xyweng@zju.edu.cn

ranges between 10 and 20 % of the total flow (Lovelock and Winter 1996). In wheat leaf under drought, the electron flux for the photoreduction of O_2 to water is about 30 % of the total flux (Biehler and Fock 1996). Miyake and Yokota (2000) estimated that the potential capacity of the water-water cycle under ambient condition might be higher than previously estimated. Makino *et al*. (2002) found oxygen reduction in the water-water cycle together with associated electron transport is also a major sink of electron. According to Schreiber and Neubauer (1990) and Neubauer and Yamamoto (1992) the water-water cycle assists in developing and maintaining a high pH gradient across the thylakoid membranes, which in turn enhances non-radiative energy dissipation.

Although the effects of P-deficiency on the non-photochemical quenching (Jacob and Lawlor 1993) and the amount of ATP (Fredeen *et al*. 1990, Jacob and Lawlor 1993) have been examined in detail, little is known about the response of water-water cycle to P-deficiency and its coordination with non-photochemical quenching and ATP amount. The aim of this work is to establish whether water-water cycle is enhanced during P-deficiency of rice and whether water-water cycle serves as an efficient electron sink; and, further, to verify that the enhanced water-water cycle maintains a high proton gradient and enhances non-radiative energy dissipation.

Materials and methods

Plants and growth condition: Seeds of rice (O*ryza sativa* L. ssp. *indica* cv. Zhenong 966) were germinated and planted according to Jiang *et al.* (1987). Plants of similar development stage (3-leaf stage) were selected for transplanting into 1 dm^3 plastic pots filled with International Rice Research Institute complete nutrient solution, containing 0.3 mM KH_2PO_4 (Jiang *et al*. 1987), and grown outdoors in a net room in Hua-jia-chi Campus of the Zhejiang University. The nutrient solutions were renewed twice a week and the pH was adjusted to 5.0 - 5.2 regularly during the cultivation period. Two weeks later, the control and P-deficient groups were grown with complete or P-free nutrient solution, respectively. All the measurements of photosynthetic parameters and chlorophyll (Chl) fluorescence were conducted on the 6th leaf of the main stem 10 d after the treatment. After the measurements, the leaves were excised, frozen in liquid N_2, and stored at -80 °C for biochemical assays.

Gas exchange and Chl fluorescence were measured simultaneously with an integrating fluorescence fluorometer (*LI-6400–40* leaf chamber fluorometer, *Li-Cor*, Lincoln, USA). Net photosynthetic rate (P_N), stomatal conductance (g_s), intercellular CO_2 concentration (c_i), and steady-state fluorescence yield (F_s) were constantly monitored to ensure that they reached steady state before a reading was taken. Maximal fluorescence under irradiation (F_m') was obtained by imposing a saturation flash (>6 000 μmol m^{-2} s^{-1}). After the flash, actinic irradiation was removed, far-red irradiation was given, and F_0' was determined. The quantum efficiency of photosystem (PS) 2 (Φ_{PS2}) is defined as $(F_m' - F_s)/F_m'$, photochemical quenching q_P is defined as $(F_m' - F_s)/(F_m' - F_0')$, and non-photochemical quenching q_N was calculated as $(F_m - F_m')/(F_m - F_0')$ as proposed by Genty *et al*. (1989). Maximal fluorescence (F_m) and minimal fluorescence (F_0) of dark-adapted leaves were measured after dark adapting the leaves for 30 min. All these measurements were performed over a range of photosynthetic photon flux densities (PPFDs) at a leaf temperature of 28 ± 2 °C, in the presence of a CO_2 concentration of 385 ± 5 cm^3 m^{-3}, and a constant ambient oxygen concentration of 21 and 2 %, respectively. PPFD was set at 0, 100, 200, 500, 1 000, 1 500, and 2 000 μmol m^{-2} s^{-1}.

Calculations of electron transport rate: The electron transport rate through PS 2 (J_f) was estimated from the fluorescence data according to the following equation: $J_f = PPFD \times \Phi_{PS2} \times \alpha$, where Φ_{PS2} is the quantum yield of PS 2 electron transport, and α is a constant that depends on the molar ratio of PS 2 to PS 1 and the efficiency of photon absorption by the leaves and it was determined according to the method of Miyake and Yokota (2000). The rate of electron transport required to maintain photosynthetic carbon reduction cycle (PCR) and photorespiration carbon oxidation cycle (PCO) was calculated from gas exchange according to Von Caemmerer and Farquhar (1981):
$J_g = (P_N + R_D)(4\ c_c + 8\ \Gamma^*)/(c_c - \Gamma^*)$ where P_N is net photosynthetic rate, R_D is rate of mitochondrial respiration in the light, c_c is the pressure of CO_2 at site of carboxylation, and Γ^* is the partial pressure of CO_2 at which the rate of carboxylation of RuBP equals to the rate of photorespiration evolution of CO_2. R_D and Γ^* were determined according to the methods of Brooks and Farquhar (1985). c_c was determined by the equation: $c_c = c_i - P_N/g_m$, where g_m is mesophyll conductance to CO_2 determined according to Harley *et al.* (1992). The rate of electron transport though photorespiration (J_o) was calculated from the equation: $J_o = 2/3\ [J_f - 4\ (P_N + R_D)]$ (Epron *et al*. 1995). The rate of alternative electron transport (J_a) was calculated from the equation: $J_a = J_f - J_g$ (Makino *et al*. 2002).

Measurement of ATP content: Extraction of ATP was performed as described by Wang *et al.* (2003). The excised leaves were cut into small pieces and extracted with boiling water for 10 min. ATP content in the solutions were measured with the luciferin-luciferase assay. The medium for measurement included 50 mM glycylglycine (pH 7.6), 10 mM $MgSO_4$ and 1 mM EDTA.

Enzyme activity assays: Leaf tissue (0.3 g) was ground in liquid nitrogen to a fine powder using a mortar and pestle. The powder was transferred to a pre-cooled (4 °C) mortar

and pestle with 6 cm^3 of 50 mM sodium phosphate buffer (pH 7.0) containing 0.1 mM EDTA. The homogenate was centrifuged at 15 000 *g* for 20 min at 4 °C. The resulting supernatant was used for determination of enzyme activity. Superoxide dismutase (SOD; EC 1.15.1.1) activity was determined spectrophotometrically according to Koca *et al*. (2006). One unit of SOD activity was defined as the amount of enzyme required to cause 50 % inhibition of the rate of NBT reduction at 560 nm. Ascorbate peroxidase (APX; EC 1.11.1.11) activity was determined according to the method of Nakano and Asada (1981). The enzyme activity was defined as the decrease of 0.01 A_{290} per minute. The protein (Pr) content in enzyme extracts was determined by the method of Bradford (1976) using bovine serum albumin as a standard.

Superoxide radical production rate was measured by monitoring the nitrite formation from hydroxylamine in the presence of $O_2^{\cdot -}$ according to Elstner and Heupel (1976). Leaf segments (about 5 g fresh mass) were homogenized using a chilled pestle and mortar in 6 cm^3 of 65 mM phosphate buffer (pH 7.8). The homogenate was filtered through 4 layers of *Miracloth*. The filtrate was centrifuged at 5 000 *g* for 10 min at 4 °C. 0.9 cm^3 of phosphate buffer and 0.1 cm^3 of 10 mM hydroxylamine hydrochloride were added to 1 cm^3 of supernatant. This mixture was incubated at 25 °C for 20 min. 0.5 cm^3 of the incubated mixture was injected into the solution containing 0.5 cm^3 of 17 mM *p*-aminobenzoic acid and 0.5 cm^3 of 17 mM α-napthaleneamine at 25 °C for 20 min. The solution was shaken with equal volume of ether and the mixture was centrifuged at 1 500×*g* for 5 min. Absorbance of the pink water phase was then measured at 530 nm.

Membrane lipid peroxidation state in the leaves was estimated using malonyldialdehyde (MDA) as an indicator (Huang *et al*. 2004). Frozen leaf blade (0.2 g) was ground into powder with the mortar and pestle in liquid N_2 and extracted with 10 cm^3 of 10 % trichloroacetic acid (TCA). The homogenate was centrifuged at 4 000 *g* for 10 min. 2 cm^3 of supernatant was added to 2 cm^3 of 0.6 % barbiturate (TBA) (the solution was solved in 10 % TCA). After keeping in boiling water bath for 20 min and immediate cooling in an ice bath, the mixture was centrifuged at 4 000 *g* for 10 min again. The absorbances of supernatant were measured at 450, 532, and 600 nm with a spectrophotometer *UV-1201* (*Shimadzu*, Kyoto, Japan). The formula of MDA determination was: MDA [μM] = 6.45 (A_{532} - A_{600}) - 0.56 A_{450}, where 450 nm was the absorption peak of soluble saccharides.

Statistical analysis: Treatment means were compared by LSD. For all analyses, differences were considered to be significant at the $P<0.05$ level. Analysis was performed using *SYSTAT* version *10.0* (*SPSS*, Chicago, USA).

Results

Effect of P-deficiency on gas exchange: In response to increased PPFD, P_N in both control and stressed leaves increased rapidly when PPFD was below 1 000 μmol m^{-2} s^{-1}, then maintained constant at PPFD between 1 000 to 1 500 μmol m^{-2} s^{-1}, and finally it began to decrease at PPFD above 1 500 μmol m^{-2} s^{-1} (Fig. 1*A*). Compared to control, P_N was significantly reduced in P-deficient leaves, decreasing by 19.5, 18.2, and 19.8 % at PPFD of 1 000, 1 500, and 2 000 μmol m^{-2} s^{-1}, respectively. Similarly, g_s was decreased by 21.1, 23.6, and 15.9 % in the stressed leaves at these irradiances (Fig. 1*B*), whereas c_i remained constant in P-deficient leaves (Fig. 1*C*).

Effects of P-deficiency on Φ_{PS2}, q_P, and q_N: Φ_{PS2}, q_P, and q_N were measured over a range of PPFDs in the presence of 21 and 2 % O_2 (Fig. 2). There was no difference in Φ_{PS2} between the control and the treated plants under 21 % O_2, however, it was significantly decreased in P-deficient plants when the O_2 concentration decreased to 2 %. This suggests that the electron transport under P-deficiency was sensitive to oxygen concentration (Fig. 2*A*). Similarly, q_P was little affected under 21 % O_2 in the plants suffering P starvation (Fig. 2*B*). In contrast q_P was significantly decreased under 2 % O_2 in P-deficient leaves. Unlike q_P, q_N was higher in P-deficient leaves than that in control under 21 % O_2 (Fig. 2*C*). At decrease of oxygen concentration from 21 to 2 %, the difference of q_N between the control and the stressed plants was diminished when PPFD exceeded 1 000 μmol m^{-2} s^{-1}.

Effects of P-deficiency on electron distribution: By simultaneous measurement of Chl fluorescence and gas exchange, we calculated the electron transport rate through PS 2 (J_f), the electron transport rate through photorespiration (J_o), and the electron transport rate required to sustain CO_2 assimilation and photorespiration (J_g). The difference between J_f and J_g was regarded as the rate of alternative electron transport (J_a), which accounts for the electron flux in the water-water cycle (Miyake and Yokota 2000). Compared with the control, J_f changed slightly in the P-deficient plants (Fig. 3*A*). However, the fraction of total electron flow partitioned to J_g was declined markedly, whereas the fraction of total electron flow partitioned to J_a was elevated in P-stressed plants (Fig. 3*B*,*D*). Meanwhile, the fraction of total electron flow partitioned to J_o was also increased. Compared to control, the ratios of J_o/J_f and J_a/J_f in P-deficient leaves were increased by 13.4 and 137.1 % at a PPFD of 1 000 μmol m^{-2} s^{-1} (Fig. 3*B*,*D*), respectively, while the ratio of J_g/J_f was decreased by 25.7 % (Fig. 3*C*).

Effects of P-deficiency on the rate of $O_2^{\cdot -}$ production, activities of SOD and APX, and contents of MDA and ATP: The limiting step in the water-water cycle is the

photoreduction of O_2 to $O_2^{\cdot-}$ (Asada 1999). The rate of $O_2^{\cdot-}$ production was increased by 32.5 % in the P-deficient leaves. SOD and APX are two of the key enzymes involved in scavenging of active oxygen species in the water-water cycle. The activities of SOD and APX were increased by 13.9 and 56.8 %, respectively, compared to control. MDA content was slightly changed, while ATP content was reduced by 24.9 % in P-deficient rice plants (Table 1).

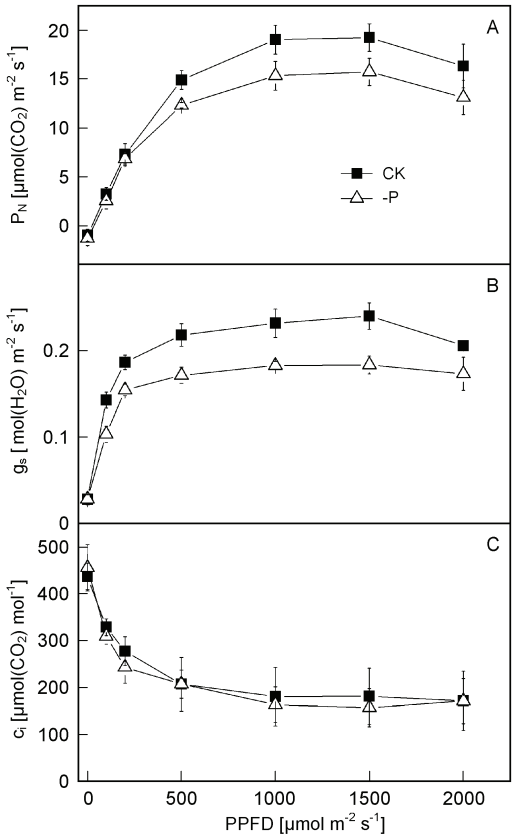

Fig. 1. Responses of net photosynthetic rate P_N (*A*), stomatal conductance g_s (*B*), and intercellular CO_2 concentration c_i (*C*) to photosynthetic photon flux density (PPFD) under P-deficiency in the leaves of rice in the presence of 21 % O_2. Means ± SE of at least three measurements of separate leaves. CK - control, -P - P-deficiency.

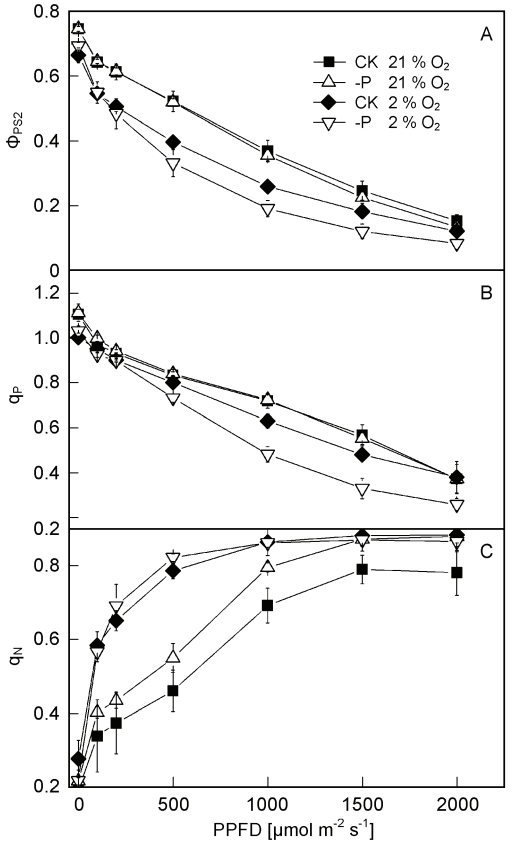

Fig. 2. Responses of photosystem 2 quantum yield Φ_{PS2} (*A*), photochemical quenching q_P (*B*), and non-photochemical quenching q_N (*C*) to photosynthetic photon flux density (PPFD) under P-deficiency in the leaves of rice in the presence of 21 % and 2 % O_2, respectively. Means ± SE of at least three measurements of separate leaves. CK - control, -P - P-deficiency.

Discussion

Both stomatal and non-stomatal limitation factors affect photosynthesis (Farquhar and Sharkey 1982). In the present experiment, P_N (Fig. 1*A*) and g_s (Fig. 1*B*) were reduced in the P-deficient plants, which indicated that stomatal limitation was the cause for photosynthesis inhibition. Besides stomatal limitation, Starck *et al*. (2000) found that the disturbance in the chloroplast photosystems was the cause for photosynthesis inhibition after P starvation. Φ_{PS2} and J_f were not significantly reduced by P-deficiency (Figs. 2*A* and 3*A*). The ratio of J_g/J_f was significantly reduced in P-deficient plants (Fig. 3*B*), but the ratio of J_o/J_f was elevated (Fig. 3*C*), indicating that the decrease of J_g was caused by the depression of CO_2 assimilation. Therefore, the decline in photosynthesis might be due to the slow-down of photosynthetic carbon reduction cycle under P-deficiency. Generally, P-deficiency depresses the content and activity of ribulose-1, 5-bisphosphate carboxylase/oxygenase, or RuBP regeneration capacity (Lauer *et al*. 1989, Rao and Terry 1989, Fredeen *et al*. 1990, Jacob and Lawlor 1992, Pieters *et al*. 2001), which might be the reason for the inhibition in photosynthetic carbon reduction cycle.

CO_2 assimilation is the major consumer for photon energy absorbed in antennae. The P-stress induced decline in CO_2 fixation implies the decreased demand for reducing equivalent (ATP and NADPH). Consequently, the absorption of photon energy exceeds what is required. This would result in the accumulation of excess excitation energy, which, if not safely dissipated, could be harmful to photosynthetic apparatus. Demmig-Adams and Adams

Table 1. The rate of $O_2^{\cdot-}$ production, activities of SOD and APX, and contents of MDA and ATP in the leaves from rice plants grown under P-sufficient conditions (CK) or P-deficient conditions (-P). Means ± SE of at least three individual measurements. Values within the same column followed by a different letter are significantly different at $P < 0.05$.

Treatment	$O_2^{\cdot-}$-production [nmol s^{-1} g^{-1}(Pr)]	SOD activity [unit mg^{-1}(Pr)]	APX activity [unit mg^{-1}(Pr)]	MDA content [μmol kg^{-1}(FM)]	ATP content [nmol g^{-1}(FM)]
CK	24.43 ± 1.56a	14.49 ± 0.63a	6.25 ± 0.62a	42.2 ± 4.27a	10.7 ± 0.30b
-P	32.36 ± 0.30b	16.50 ± 0.30b	9.81 ± 0.82b	43.4 ± 4.17a	8.0 ± 0.38a

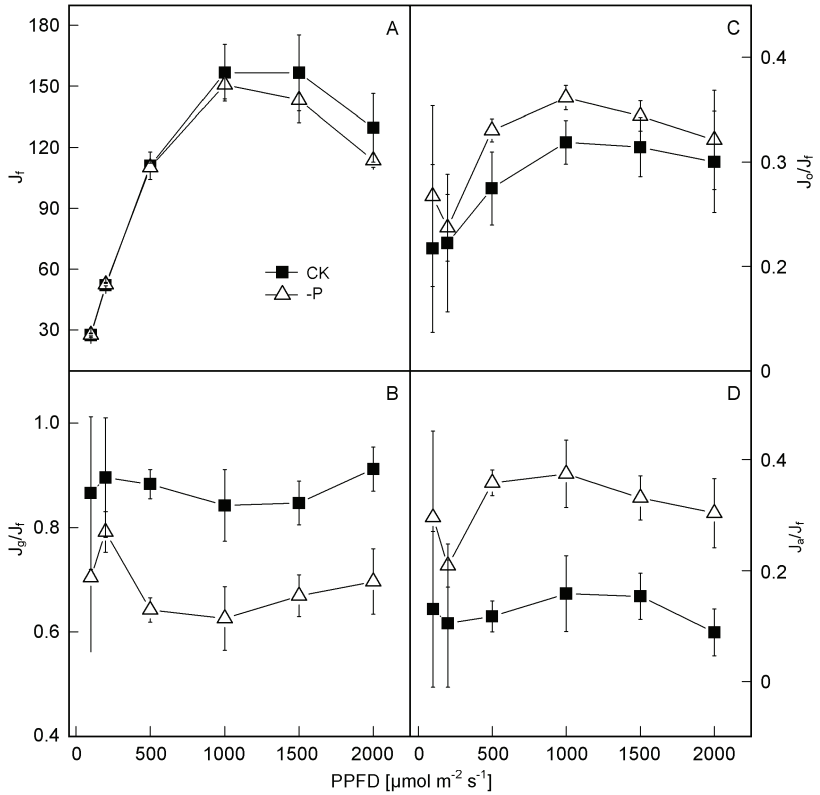

Fig. 3. Responses of J_f (*A*), and the ratios of J_g/J_f (*B*), J_o/J_f (*C*), and J_a/J_f (*D*) to photosynthetic photon flux density (PPFD) under P-deficiency in the leaves of rice at 21 % O_2. Means ± SE of at least 3 measurements of separate leaves. CK - control, -P - P-deficiency.

(1992) proposed that non-radiative dissipation of excitation energy occurs in plants as an internal mechanism for protection of photosynthetic apparatus. Our results that q_N increased in P-deficient plants confirmed an important role of non-radiative energy dissipation in photoprotection in response to the stress of P-deficiency.

Excess excitation energy often induces alternative routes for electron transport. In our study, compared to the control, the electron transport rate through PS 2 (J_f) changed only little (Fig. 3*A*). This was in accordance with the conclusions of Abadia *et al*. (1987) and Milivojević *et al*. (2006). However, the electron transport rate required to sustain CO_2 assimilation and photorespiration (J_g) was dramatically reduced in the P-deficient plants (Fig. 3*B*), which indicated that some alternative routes for electron transport did exist. At 21 % O_2 concentration, no statistical difference in Φ_{PS2} was observed between the control and P-stressed plants. As O_2 concentration decreased from 21 to 2 %, Φ_{PS2} declined faster in P-deficient plants than in control (Fig. 2*A*), suggesting a larger proportion of O_2-mediated electron transport was involved in P-deficiency. The prime candidates involved in O_2-mediated electron consumption are water-water cycle and photorespiration, which can serve as a sink to drain the excess electrons off the electron chain. In our case, the ratio of J_o/J_f increased in P-deficient plants in comparison with the control (Fig. 3*C*), demonstrating that photo-respiration was up-regulated in response to P-stress. We also discovered that water-water cycle was significantly enhanced by the prominent increase in the ratio of J_a/J_f (Fig. 3*D*). Moreover, the increase in J_a/J_f was much greater

than that of J_o/J_f. These results confirmed that water-water cycle was more sensitive to P-deficiency than photorespiration. It also revealed that water-water cycle could operate as an efficient electron sink for excess excitation energy.

In addition to acting as a sink for excess electrons, water-water cycle contributes to generation of proton gradient across thylakoid membrane (Schreiber and Neubauer 1990, Neubauer and Yamamoto 1992). Since phosphorus is a component of ATP, P-limitation will lead to the reduction of ATP synthesis (Fredeen *et al*. 1990, Jacob and Lawlor 1993). In this experiment, ATP content was decreased by 24.9 % when rice plants suffered P starvation for 10 d (Table. 1). The decrease in ATP synthesis would result in little proton consumption, which in turn contributes to accumulation of the proton gradient across the thylakoid membrane. This thylakoid acidification is necessary for the non-radiative dissipation of excess energy. We found that q_N in P-deficient plants was higher than that in the control at 21 % O_2, while the difference disappeared when water-water cycle was inhibited at 2 % O_2. This confirmed that water-water cycle is involved in non-radiative dissipation of excess energy in P-limitation stress.

Water-water cycle, however, is inevitably coupled with the generation of reactive oxygen species (ROS), such as $O_2^{\cdot-}$ and H_2O_2 (Asada 1999), which are potentially harmful to photosynthetic apparatus. However, the activities of the antioxidant enzymes are usually up-regulated correspondingly for scavenging ROS in plants subjected to stress (Agarwal and Pandey 2004, Ghorbanli *et al*. 2004, Rahnama and Ebrahimzadeh 2005). In this study, the increased $O_2^{\cdot-}$ production rate was accompanied by significantly elevated activities of SOD and APX (Table. 1). Moreover, MDA content in P-limited plants did not show significant increase (Table. 1), which indicated that the membrane lipid peroxidation was not aggravated when the plants suffered P-starvation for 10 d.

To summarize, we found that electron flow in the water-water cycle was enhanced in P-deficient plants. It served as an efficient sink for excess electrons and helped to dissipate excess photon energy as non-radiative dissipation. Therefore, water-water cycle was an important photoprotective mechanism for adapting the photosynthetic apparatus to P-deficiency in rice.

References

Abadia, J. Rao, I.M., Terry, N: Changes in leaf phosphate status have only small effects on the photochemical apparatus of sugar beet leaves. - Plant Sci. **50**: 49-55, 1987.

Agarwal, S., Pandey, V.: Antioxidant enzyme responses to NaCl stress in *Cassia angustifolia*. - Biol. Plant. **48**: 555-560, 2004.

Asada, K.: The water-water cycle in chloroplasts: Scavenging of active oxygens and dissipation of excess photons. - Annu. Rev. Plant Physiol. - Plant mol. Biol. **50**: 601-639, 1999.

Biehler, K., Fock, H.: Evidence for the contribution of the Mehler-peroxidase reaction in dissipating excess electrons in drought-stressed wheat. - Plant Physiol. **112**: 265-272, 1996.

Bradford, M.M.: A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. - Anal. Biochem. **72**: 248-254, 1976.

Brooks, A., Farquar, G.D.: Effects of temperature on the CO_2/O_2 specificity of ribulose-1,5-bisphosphate carboxylase/ oxygenase and the rate of respiration in the light. - Planta **165**: 397-406, 1985.

Demmig-Adams, B., Adams, W.W., III: Photoprotection and other responses of plants to high light stress. - Annu. Rev. Plant Physiol. Plant mol. Biol. **43**: 599-626, 1992.

Elstner, E.F., Heupel, A.: Inhibition of nitrite formation from hydroxylammonium-chloride simple assay for superoxide dismutase. - Anal. Biochem. **70**: 616-620, 1976.

Epron, D., Godard, D., Cornic, G., Genty, B.: Limitation of net CO_2 assimilation rate by internal resistance to CO_2 tranfer in the leaves of two tree species (*Fagus sylvation* L. and *Castanea sativa* Mill). - Plant Cell Environ. **18**: 43-51, 1995.

Farquhar, G.D., Sharkey, T.D.: Stomatal conductance and photosynthesis. - Annu. Rev. Plant Physiol. **33**: 317-345, 1982.

Fredeen, A.L., Raab, T.K., Rao, I.M., Terry, N.: Effects of phosphorus nutrition on photosynthesis in *Glycine max* L. Merr. - Planta **181**: 399-405, 1990.

Genty, B., Briantais, J.M., Baker, N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. - Biochim. biophys. Acta. **990**: 87-92, 1989.

Ghorbanli, M., Ebrahimzadeh, H., Sharifi, M.: Effects of NaCl and mycorrhizal fungi on antioxidative enzymes in soybean. - Biol. Plant. **48**: 575-581, 2004.

Harley, P.C., Poreto, F., Marco, G.D., Sharkey, T.D.: Theoretical considerations when estimating the mesophyll conductance to CO_2 flux by analysis of the response of photosynthesis to CO_2. - Plant Physiol. **98**: 1429-1436, 1992.

Huang, Z.A., Jiang, D.A., Yang, Y., Sun, J.W., Jin, S.H.: Effects of nitrogen deficiency on gas exchange, chlorophyll fluorescence, and antioxidant enzymes in leaves of rice plants. - Photosynthetica **42**: 357-364, 2004.

Jacob, J., Lawlor, D.W.: Stomatal and mesophyll limitations of photosynthesis in phosphate deficient sunflower, maize and wheat plants. - J. exp. Bot. **42**: 1003-1011, 1991.

Jacob, J., Lawlor, D.W.: Dependence of photosynthesis of sunflower and maize leaves on phosphate supply, ribulose-1,5-bisphosphate carboxylase/oxygenase activity, and ribulose-1,5-bisphosphate pool size. - Plant Physiol. **98**: 801-807, 1992.

Jacob, J., Lawlor, D.W.: *In vivo* photosynthetic electron transport does not limit photosynthetic capacity in phosphate-deficient sunflower and maize leaves. - Plant Cell Environ. **16**: 785-795, 1993.

Jiang, D.A., Rao, L.H., Peng, Z.Q.: [Some physiological effects of potassium on yield formation of rice.] - Acta Agr. Univ. Zhejiang **13**: 441-444, 1987. [In Chinese.]

Koca, H., Ozdemir, F., Turkan I.: Effect of salt stress on lipid peroxidation and superoxide dismutase and peroxidase activities of *Lycopersicon esculentum* and *L. pennellii*. - Biol. Plant. **50**: 745-748, 2006.

Lauer, M.J., Pallardy, S.G., Belvins, D.G., Randall, D.D.: Whole leaf carbon exchange characteristics of phosphate deficient soybeans (*Glycine max* L.). - Plant Physiol. **91**: 848-854, 1989.

Lovelock, C.E., Winter, K.: Oxygen-dependent electron transport and protection from photoinhibition in leaves of tropical trees species. - Planta **198**: 580-587, 1996.

Makino, A., Miyake, C., Yokota, A.: Physiological function of the water-water cycle (Mehler reaction) and the cyclic electron flow around PS1 in rice leaves. - Plant Cell Physiol. **43**: 1017-1026, 2002.

Maleszewski, S., Clereszko, I., Skowrońska, A., Mieczejko, E., Kozłowska-Szerenos, B.: Changes induced by low oxgen concentration in photosynthetic and respiratory CO_2 exchange in phosphate-deficient bean leaves. - Biol. Plant. **48**: 401-405, 2004.

Mehler, A.H.: Studies on reactions of illuminated chloroplasts. I. Mechanism of the reduction of oxygen and other Hill reagents. - Arch. Biochem. Biophys. **33**: 65-77, 1951.

Milivojević, D.B., Nikolić, B.R., Drinić, G.: Effects of arsenic on phosphorus content in different organs and chlorophyll fluorescence in primary leaves of soybean. - Biol. Plant. **50**: 149-151, 2006.

Miyake, C., Yokota, A.: Determination of the rate of photoreduction of O_2 in the water-water cycle in watermelon leaves and enhancement of the rate by limitation of photosynthesis. - Plant Cell Physiol. **42**: 508-515, 2000.

Nakano, Y., Asada, K.: Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. - Plant Cell Physiol. **22**: 867-880, 1981.

Neubauer, C., Yamamoto, H.Y.: Mehler-peroxidase reaction mediates zeaxanthin formation and zeaxanthin-related fluorescence quenching in intact chloroplasts. - Plant Physiol. **99**: 1354-1361, 1992.

Niyogi, K.K.: Photoprotection revisited: genetic and molecular approaches. - Annu. Rev. Plant Physiol. Plant mol. Biol. **50**: 333-359, 1999.

Niyogi, K.K.: Safety valves for photosynthesis. - Curr. Opinion Plant Biol. **3**: 455-460, 2000.

Pieters, A.J., Paul, M.J., Lawlor, D.W.: Low sink demand limits photosynthesis under Pi deficiency. - J. exp. Bot. **52**: 1083-1091, 2001.

Qiu, I., Israel, D.W.: Carbohydrate accumulation and utilization in soybean plants in response to altered phosphorus nutrition. - Physiol. Plant. **90**: 722-728, 1994.

Rahnama, H., Ebrahimzadeh, H.: The effect of NaCl on antioxidant enzyme activities in potato seedlings. - Biol. Plant. **49**: 93-97, 2005.

Rao, I.M., Terry, N.: Leaf phosphate status, photosynthesis and carbon partitioning in sugar beet. I. Changes in growth, gas exchange and Calvin cycle enzymes. - Plant Physiol. **90**: 814-819, 1989.

Schreiber, U., Neubauer, C.: O_2-dependent electron flow, membrane energization and the mechanism of non-photochemical quenching of chlorophyll fluorescence. - Photosynth. Res. **25**: 279-293, 1990.

Starck, Z,, Niemyska, B., Bogdan, J., Akour Tawalbeh, R.N.: Response of tomato plants to chilling stress in association with nutrient or phosphorus starvation. - Plant Soil **226**: 99-106, 2000.

Von Caemmerer, S., Farquhar, G.D.: Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. - Planta **153**: 376-387, 1981.

Wang, H.W., Mi, H., Ye, J.Y., Deng, Y., Shen, Y.K.: Low concentrations of $NaHSO_3$ increase cyclic photo-phosphorylation and photosynthesis in cyanobacterium *Synechocystis* PCC6803. - Photosynth. Res. **75**: 151-159, 2003.