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RESEARCH PAPER

Inhibition of photosynthesis and energy dissipation induced by water and high light stresses in rice

Yanhong Zhou[1,2], Hon Ming Lam[3] and Jianhua Zhang[2,*]

[1] *Department of Horticulture, Zhejiang University, Hangzhou, China*

[2] *Department of Biology, Hong Kong Baptist University, Hong Kong, China*

[3] *Department of Biology, The Chinese University of Hong Kong, Hong Kong, China*

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Abstract

Photoprotection mechanisms of rice plants were studied when its seedlings were subjected to the combined stress of water and high light. The imposition of water stress, induced by PEG 6000 which was applied to roots, resulted in substantial inhibition of stomatal conductance and net photosynthesis under all irradiance treatments. Under high light stress, the rapid decline of photosynthesis with the development of water stress was accompanied by decreases in the maximum velocity of RuBP carboxylation by Rubisco (V_{cmax}), the capacity for ribulose-1,5-*bis*phosphate regeneration (J_{max}), Rubisco and stromal FBPase activities, and the quantum efficiency of photosystem II, in the absence of any stomatal limitation of CO_2 supply. Water stress significantly reduced the energy flux via linear electron transport (J_{PSII}), but increased light-dependent and ΔpH- and xanthophyll-mediated thermal dissipation (J_{NPQ}). It is concluded that the drought-induced inhibition of photosynthesis under different irradiances in the rice was due to both diffusive and metabolic limitations. Metabolic limitation of photosynthesis may be related to the adverse effects of some metabolic processes and the oxidative damage to the chloroplast. Meanwhile, an enhanced thermal dissipation is an important process to minimize the adverse effects of drought and high irradiance when CO_2 assimilation is suppressed.

Key words: Light stress, *Oryza sativa*, photosynthesis, photoprotection, photoinhibition, rice, water stress.

Introduction

Drought is considered to be a major environmental factor limiting plant growth and yield worldwide, especially in arid and semi-arid areas (Boyer, 1982). It induces many physiological, biochemical, and molecular responses in which photosynthesis is one of the primary physiological targets (Chaves, 1991; Lawlor, 1995). There is a long-standing controversy as to whether drought primarily limits photosynthesis through stomatal closure (Sharkey, 1990; Chaves, 1991; Ort *et al*., 1994) and, in general, through reduced mesophyll conductance (Massacci and Loreto, 2001; Centritto *et al*., 2003; Flexas *et al*., 2004) or metabolic impairment (Boyer, 1976; Lawlor, 1995). In recent years, a suggestion about ATP limitation of photosynthesis under mild water stress (Tezara *et al*., 1999), has further stimulated the debate (Cornic, 2000; Flexas and Medrano, 2002*a*; Lawlor and Cornic, 2002; Tezara *et al*., 2002). Generally, during the onset of the water stress, stomatal limitation is responsible for the decline of photosynthesis (Chaves, 1991; Cornic, 2000).

* To whom correspondence should be addressed. E-mail: jzhang@hkbu.edu.hk

Abbreviations: A_{max}, light- and CO_2-saturated rate of CO_2 assimilation; A_{sat}, light-saturated rate of CO_2 assimilation at an ambient CO_2 concentration of 360 μmol mol^{-1}; F_o, F_m, relative Chl fluorescence yield for open and closed PSII reaction centres, respectively, in dark-treated samples; F_o', minimum fluorescence yield corresponding to open reaction centres during illumination; F_s, F_m', steady-state or maximum fluorescence yield during illumination, respectively; F_v/F_m, intrinsic quantum efficiency of PSII photochemistry; I_A, photosynthetically active radiation absorbed by the leaf; J_{max}, maximum potential rate of electron transport contributed to RuBP regeneration; *I*, stomatal limitation; *NPQ*, non-photochemical quenching; MDA, malondialdehyde; MSI, membrane stability index; PSII, photosystem II; V_{cmax}, maximum velocity of RuBP carboxylation by Rubisco; $\Phi_{f,D}$, $J_{f,D}$, combined quantum efficiency or flux of fluorescence and constitutive thermal dissipation, respectively; Φ_{NF}, J_{NF}, quantum yield or flux of thermal dissipation in non-functional PSII, respectively; Φ_{NPQ}, J_{NPQ}, quantum yield or flux of light-dependent and ΔpH- and xanthophyll-mediated regulated thermal dissipation, respectively; Φ_{PSII}, J_{PSII}, quantum yield or flux of PSII electron flow, respectively; *qP*, photochemical quenching coefficient.

With the progress of water stress and tissue dehydration, a gradual metabolic impairment will arise (Kaiser, 1987), including the limitation of photophosphorylation (Younis *et al*., 1979; Tezara *et al*., 1999), RuBP regeneration (Giménez *et al*., 1992; Gunasekera and Berkowitz, 1993), and Rubisco activity (Maroco *et al*., 2002; Parry *et al*., 2002). However, metabolic limitation of photosynthesis was always overestimated according to the traditional methods for A/C_i analysis (Flexas *et al*., 2004). Recently, alternative methods, taking into account variations in mesophyll conductance, have been proposed (Ethier and Livingston, 2004; Manter and Kerrigan, 2004).

Although plant responses to drought are relatively well known, plant performance under a more complex environment where stresses are far from single is fragmentary. This is due, at least in part, to the fact that plant response to simultaneous stresses (e.g. drought, excessive light and heat) which may have a common occurrence in the field is usually not predictable from single factor studies. For example, plants often absorb more light energy than they need for photosynthesis under these stress conditions, as the capacity of the reactions that convert solar energy into chemical energy is limited, and excessive light absorption exacerbates the inactivation of PSII under drought (Björkman and Powles, 1984; Valladares and Pearcy, 1997). To protect the chloroplast from surplus energy damage during drought, plants must have developed tolerance mechanisms to dispose of them rapidly. Thermal dissipation of light energy, absorbed in excess (measured as non-photochemical quenching, *NPQ*) by the light-harvesting antenna complex of PSII, is believed to be one of the most important of the rapidly activated regulatory mechanisms (Holt *et al*., 2004; Horton *et al*., 2005; Lavaud and Kroth, 2006). In higher plants, *NPQ* is triggered by the light-driven build-up of a transthylakoid proton gradient (ΔpH), in which both the protonation of the light-harvesting antenna complex and the production of de-epoxidized xanthophylls are involved (Lavaud and Kroth, 2006). The enhancement of photoprotective capacity, which competes with photochemistry for the absorbed energy, results in a down-regulation of photosynthesis which is shown by the decrease in quantum yield of PSII (Genty *et al*., 1989). However, it has been documented, in plants native to semi-arid regions, that electron transport associated with O_2 uptake processes (photorespiration and the Mehler reaction) increased, which presumably maintain a high ΔpH and *NPQ*, dissipating excess light as heat and providing photoprotection to the photosynthetic apparatus (Harbinson *et al*., 1990; Biehler and Fock, 1996; Flexas *et al*., 1999; Wingler *et al*., 1999).

In spite of numerous reports about the effects of water deficit on dryland plants, drought tolerance and responses of rice as a wetland plant remain poorly understood. It is true that rice normally grows in paddy field conditions where monsoons and irrigation facilities supply abundant water. Nevertheless, rice yield is often substantially reduced due to periodic drought in the rainfed lowland ecosystem in South and Southeast Asia (Wade *et al*., 1995; Cabuslay *et al*., 2002). In addition, water shortage in many traditional rice-grown areas has led to the demand for less irrigation. To understand how tolerant rice can be to drought or less irrigation has become an interesting issue. In addition, rice is grown in the summer and drought stress must occur simultaneously with possible light and heat stresses. In this study, the response of photosynthetic metabolism to drought stress combined with different irradiance levels was examined in rice leaves, and how its photosynthetic apparatus copes with the excessive energy input was investigated. In addition, the energy allocation of absorbed light and the membrane oxidation were analysed with relevance to the photoprotection.

Materials and methods

Plant materials

Rice (*Oryza sativa* L. subsp. *indica* cv. 9311) seeds were allowed to germinate and grow at 28 °C in a growth chamber with a photosynthetic photon flux density of 400–500 μmol m^{-2} s^{-1}. The seeds were first sown in sand and supplied with Hoaglands nutrition solution (pH 6.5). Seedlings (18-d-old) were then transferred into containers (40 cm×25 cm×15 cm) filled with the same nutrient solution. The environmental conditions were as follows: a 12 h photoperiod temperature of 25/17 °C (day/night), and photosynthetic photon flux density of 500 μmol m^{-2} s^{-1}.

Water deficit and high light stress

Water deficit was induced by PEG 6000 treatments for 2 d. The roots were directly put into PEG 6000 solutions at various concentrations. For the lighting stress, 1/3 of the plants at tillering stage were exposed to 100 μmol m^{-2} s^{-1} at 25–28 °C (LL, low light), 1/3 of the plants were exposed to 800 μmol m^{-2} s^{-1} at 25–28 °C (HL, high light), and the other 1/3 were maintained at 500 μmol m^{-2} s^{-1} with the same temperature (ML, medium light).

Gas exchange analysis

An infrared gas analyser (Ciras-1, PP system, UK) was used to estimate the light-saturated photosynthetic rate (A_{sat}), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i). The air temperature, light intensity, CO_2 concentration, and air relative humidity were maintained at 25 °C, 1000 μmol m^{-2} s^{-1}, 350 μmol mol^{-1}, and 80%, respectively.

Assimilation versus intercellular CO_2 concentration (A/C_i) curves were obtained with short-term measurements (+8 min for each data point), starting at $[CO_2]$ of 360 μmol mol^{-1} and progressively reducing the $[CO_2]$ to 0 μmol mol^{-1}; then, the $[CO_2]$ was progressively increased up to 2000 μmol mol^{-1}. The air temperature, light intensity, and air relative humidity were maintained at 25 °C, 1000 μmol m^{-2} s^{-1}, and 80%, respectively. Non-linear regression techniques, based on the equations of Harley *et al.* (1992) and Manter and Kerrigan (2004), were used to estimate the maximum carboxylation velocity of Rubisco (V_{cmax}) and maximum potential rate of electron transport contributed to RuBP regeneration (J_{max}), in which mesophyll conductance (g_i) is taken into account for the calculation of V_{cmax} and J_{max}.

Polynomials of the form $y=a+bx+cx^2$ allowed photosynthetic rates to be calculated for any given C_i, thus allowing separation of mesophyll and stomatal limitations of photosynthesis (Keiller and Holmes, 2001). Stomatal limitation (l), the proportion of photosynthesis that is limited by stomatal conductance, is calculated from equation (1)

$$l = (A_0 - A)/A_0 \quad (1)$$

where A_0 is the assimilation rate at a C_i of 360 μmol mol^{-1}, and A is the assimilation rate at an ambient external atmospheric CO_2 concentration of 360 μmol mol^{-1} (Farquahar and Sharkey, 1982).

Energy fluxes determination

Chlorophyll fluorescence was measured with a PAM-2000 chlorophyll fluorescence system under atmospheric conditions (Heinz Walz, Effieltrich, Germany). Leaves were maintained in darkness for 20 min prior to measurement of F_v/F_m. Minimal fluorescence (F_o) was measured under a weak modulating light, and maximal fluorescence (F_m) was induced by a saturating pulse of light (8000 μmol m^{-2} s^{-1}) applied over 0.8 s. The maximal quantum efficiency of PSII was determined as F_v/F_m, where F_v is the difference between F_o and F_m. An actinic light source (500 μmol m^{-2} s^{-1}) was then applied to achieve steady-state photosynthesis and to obtain F_s (steady-state fluorescence yield), after which a second saturation pulse was applied for 0.8 s to obtain F_m' (light-adapted maximum fluorescence). The actinic light was removed and the minimal fluorescence level in the light-adapted state (F_o') was determined by illuminating the leaf with a 3 s far-red light. All measurements of F_o and F_o' were performed with the measuring beam set to a frequency of 600 Hz, whereas all measurements of F_m and F_m' were performed with the measuring beam automatically switching to 20 kHz during the saturating flash. Fluorescence parameters were calculated by PAM-2000 based on the dark-adapted and light-adapted fluorescence measurements.

The quantum efficiency of photochemical energy dissipation [Φ_{PSII}, $(1 - F_s/F_m')/(F_v/F_{mM})$], thermal dissipation in photoinactivated, non-functional PSIIs [Φ_{NF},$1–(F_v/F_m)/(F_v/F_{mM})$], light-regulated thermal dissipation in active PSIIs [Φ_{PSII}, $(F_s/F_m' - F_s/F_m)(F_v/F_m)/(F_v/F_{mM})$] and a combined flux of fluorescence and constitutive, light-independent thermal dissipation ($\Phi_{f,D}$) were calculated according to Hendrickson *et al*. (2005) with $\Phi_{PSII}+\Phi_{NF}+\Phi_{NPQ}+\Phi_{f,D}=1$, where F_v/F_{mM} is F_v/F_m in non-photoinhibited leaves. The flux of energy dissipation via each process (J_{PSII}; J_{NF}; J_{NPQ}; $J_{f,D}$) was calculated by multiplying the respective quantum efficiency (Φ) with irradiance and coefficient α, respectively (Harley *et al*., 1992; Hendrickson *et al*., 2005), where α is $I_A \times 0.5$ where 0.5 is the assumed proportion of absorbed quanta used by PSII reaction centres (Melis *et al*., 1987) and I_A is the absorbed irradiance assuming an average leaf absorptance of 0.85.

Plant water status

Leaf water relations were measured at the end of gas exchange measurements. The leaves were cut, and relative water content (*RWC*) and leaf water potential (ψ_{leaf}) were determined as described by Ghannoum *et al*. (2002). The leaf relative water content was determined as follows:

$$RWC = (fresh\,weight - dried\,weight)/(fully\,turgid\,weight - dried\,weight)$$

To determine the fully turgid weight, leaves were kept in distilled water in darkness at 4 °C to minimize respiration losses until they reached a constant weight (full turgor, typically after 12 h). Leaf dry weight was obtained after 48 h at 70 °C in an oven. Five to six replicates were obtained per treatment.

Rubisco, FBPase, NADP-MDH activity determination

To induce the Rubisco, FBPase, and NADP-MDH activity, the plants were transferred to the same irradiance conditions (500 μmol m^{-2} s^{-1}) for 20 min before taking samples. Frozen leaf samples were homogenized using a chilled pestle and mortar with cooled extraction buffer containing 50 mM TRIS-HCl (pH 7.5), 1 mM EDTA, 1 mM $MgCl_2$, 12.5% (v/v) glycerine, 10% PVP, and 10 mM β-mercaptoethanol. The homogenate was centrifuged at 15 000 *g* for 15 min at 4 °C.

Rubisco activity was measured spectrophotometrically by coupling 3-phosphoglyceric acid formation with NADH oxidation at 25 °C, following Lilley and Walker (1974), with some modifications (Nakano *et al*., 2000). Initial Rubisco activity measurements were taken in a 0.1 ml reaction medium containing 5 mM HEPES–NaOH (pH 8.0), 1 mM $NaHCO_3$, 2 mM $MgCl_2$, 0.25 mM DTT, 0.1 mM EDTA, 1 U creatine phosphokinase, 1 U 3-phosphoglyceric phosphokinase, 1 U glyceraldehyde 3-phosphate dehydrogenase, 0.5 mM ATP, 0.015 mM $NADH_2$, 0.5 mM phosphocreatine, 0.06 mM RuBP, and 10 μl leaf extract. The change in absorbance at 340 nm was monitored for 90 s.

FBPase activity was determined by monitoring the increase in A_{340} using an extinction coefficient of 6.2 mM^{-1} cm^{-1} (Scheibe *et al*., 1986). Initial activity was assayed immediately after homogenization. Total activity was assayed on aliquots of enzyme extract incubated for 20 min with 100 mM dithiothreitol, 2 mM Fru-1,6-*bis*P, 10 mM $MgCl_2$, and 0.1 M HEPES-NaOH (pH 8.0). The assay mixture for initial and total activities, maintained at 25 °C, consisted of 0.1 M HEPES-NaOH (pH 8.0), containing 0.5 mM Na_2EDTA, 10 mM $MgCl_2$, 0.3 mM $NADP^+$, 0.6 mM Fru-1,6-*bis*P, 0.6 U Glc-6-P dehydrogenase from bakers' yeast (Sigma), 1.2 U Glc-P-isomerase from bakers' yeast (Sigma), and 100 μl of enzyme extract in a final volume of 1 ml. The reaction was initiated by the addition of enzyme extract.

Initial and total NADP-dependent malate dehydrogenase (NADP-MDH) activity was measured in the same leaf extracts as described in Leegood *et al*. (1982) by following the oxidation of NADP in a reaction mixture (1 ml) containing leaf extract, 0.2 mM NADPH, 0.5 mM oxaloacetate, 1 mM EDTA, 10 mM $MgCl_2$, and 0.1 M HEPES-NaOH (pH 8.0). Maximum activation was achieved by incubation of extract with 100 mM dithiothreitol in 0.1 M HEPES-NaOH (pH 8.0) for 20 min at 25 °C prior to assay.

Membrane stability, lipid peroxidation, and H_2O_2 content

The leaf membrane stability index (MSI) was determined according to the method of Sairam and Saxena (2000). Leaf discs (0.1 g) were placed in 10 ml of double-distilled water at 40 °C for 30 min and its electrical conductivity recorded using a conductivity bridge (C_1). Subsequently the same samples were kept in a boiling water bath (100 °C) for 10 min and its electrical conductivity also recorded (C_2). MSI was calculated as: MSI (%)=$[1–(C_1/C_2)]\times 100$.

The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) content, a product of lipid peroxidation. A leaf sample (0.5 g) was homogenized in 3 ml of potassium phosphate buffer (pH 7.8). The homogenate was centrifuged at 12 000 *g* for 20 min. To a 1.0 ml aliquot of the supernatant, 3.0 ml of 0.5% thiobarbituric acid in 20% TCA was added. The mixture was heated at 95 °C for 30 min and then quickly cooled in an ice bath. After centrifugation at 3000 *g* for 10 min, the absorbency of the supernatant was recorded at 532 nm. The value for non-specific absorption at 600 nm was subtracted. The MDA content was calculated using its extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as μmol MDA g^{-1} dry weight.

The H_2O_2 content was assayed with 80% acetone and was measured by monitoring the A_{410} of titanium–peroxide complex following Patterson *et al.* (1984). Plant material (1 g) was homogenized in 5 ml of cold acetone. To the whole extract, titanium reagent was added, followed by concentrated ammonium solution to precipitate the peroxide–titanium complex. After centrifugation for 5 min at 5000 *g*, the supernatant was discarded and the precipitate was dissolved in 5 ml of 2 M H_2SO_4. It was recentrifuged to remove the undissolved material and absorbance was recorded at 415 nm against a blank. The concentration of H_2O_2 was determined using a standard curve plotted with a known concentration of H_2O_2.

Statistical analysis

The results are reported as average ±standard error. The significance of results was checked by using Student's *t* test. The probability of all experiments was assumed to be $P < 0.01$.

Results

Water status of leaves

Figure 1 shows the changes in water status in rice leaf tissues during the treatment with PEG 6000. The *RWC* (%) decreased almost linearly with the increase of PEG concentration, especially under high light conditions. Meanwhile, the decreased *RWC* of leaves in PEG-treated plants was accompanied by a significant decrease in water potential (ψ_{leaf}). The correlation analysis showed that there was a strong, linear relationship between *RWC* and ψ_{leaf} for the three irradiances ($R=0.97$, $P < 0.01$, $n=54$).

Down-regulation of photosynthesis under PEG treatment

With increasing PEG concentration in the rooting media, CO_2 assimilation and g_s were gradually and significantly inhibited for all cases of lighting treatments (Fig. 2). Under the water deficit, assimilation rates and stomatal conductance decreased, especially under high light. Meanwhile, C_i decreased initially and then increased, indicating that both stomatal and non-stomatal limitations were responsible for the inhibition of photosynthesis. The increases of C_i were first found in HL leaves. When the PEG concentration is below 15% under low light, or 10% under medium and high light, increasing CO_2 supply could return A_{max} to that of the unstressed leaves, but this did not occur with increasing PEG concentration further, indicating an inhibition of photosynthetic capacity under severe drought.

Analyses of the A/C_i curves allowed the calculation of V_{cmax}, J_{max}, and l (Fig. 3A–C). V_{cmax} began to decline when PEG concentration was above 15% for LL plants, but 10% for ML and HL plants. After 2 d of 30% PEG treatment, V_{cmax} was only 25.2% for LL plants, 11.7% for ML plants, and 5.2% for HL plants, respectively, when compared with the control plants. Similar trends were found in J_{max}. A significant increase to the low concentration PEG and then a decrease to high concentration of PEG in l were observed in the experiment.

Energy flux allocation

The allocation of photons absorbed by PSII antenna to photosynthetic electron transport and thermal dissipation was assessed from the flux via linear electron transport (J_{PSII}), ΔpH- and xanthophyll-regulated thermal energy dissipation (J_{NPQ}), inactive PS II centres (J_{NF}), and fluorescence and constitutive thermal dissipation ($J_{f,D}$) which is intrinsic to the structural characteristics of the PSII light-harvesting system. For non-droughted plants, there were no significant differences in $J_{PS2,}$ J_{NPQ}, J_{NF}, and $J_{f,D}$ among the different irradiance treatments (Fig. 4). Drought resulted in a decrease in J_{PSII} and an increase in J_{NPQ} and J_{NF}, especially for HL plants. The changes became more obvious under more severe drought and higher lighting conditions. By comparison, $J_{f,D}$ was relatively constant for all the treatment combinations, indicating compensatory changes in J_{PSII}, J_{NPQ}, and $J_{N,F}$.

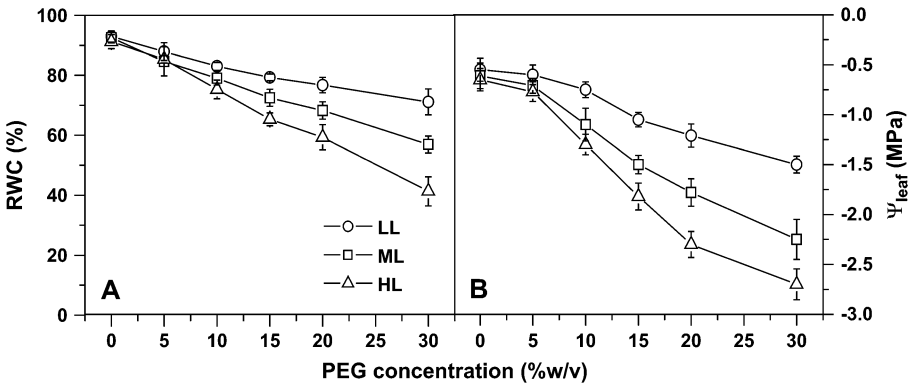

Fig. 1. Effects of 2 d exposure to PEG treatment under low light (LL), medium light (ML), and high light (HL) on relative water content [RWC (%), A] and leaf water potential (ψ_{eaf}, B) in rice leaves. Circles, LL; squares, ML; triangles, HL. Data are the means of four replicates with standard errors shown by vertical bars.

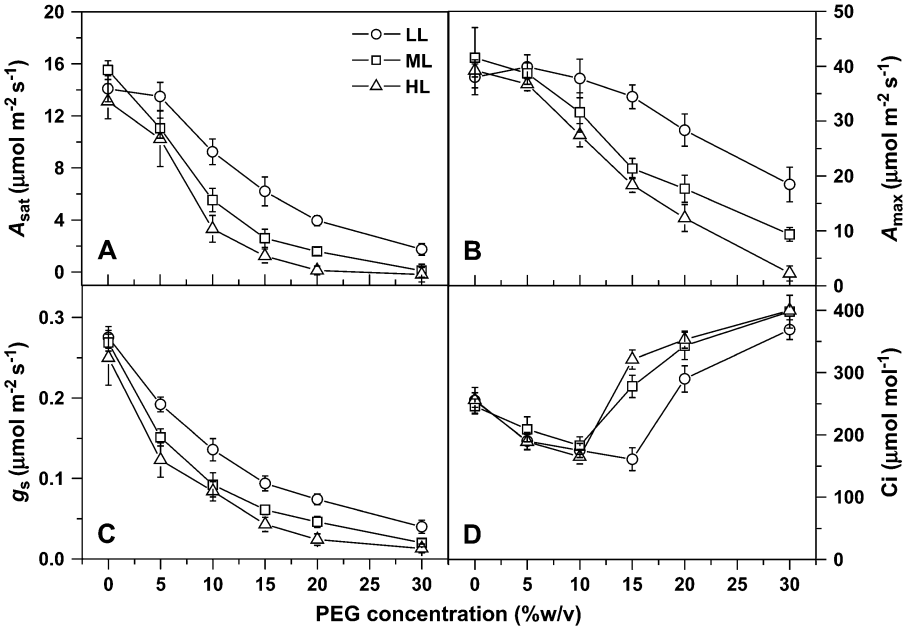

Fig. 2. Effects of 2 d exposure to PEG treatment under low light (LL), medium light (ML), and high light (HL) on light-saturated CO_2 assimilation rate (A_{sat}, A) and maximum CO_2 assimilation (A_{max}, B), stomatal conductance (g_s, C), and intercellular CO_2 concentration (C_i, D) in rice leaves. All measurements were made at 25 °C, and 1000 μmol m^{-2} s^{-1}, at a [CO_2] 360 μmol mol^{-1} (A, C, D) or 2000 μmol mol^{-1} (B). Circles, LL; squares, ML; triangles, HL. Data are the means of four replicates with standard errors shown by vertical bars.

Stromal enzyme activities

Severe drought (30% PEG) significantly reduced the activities or activation states of Rubisco (Fig. 5A–C), especially under high light. For example, initial Rubisco activity, total Rubisco activity, and activation states of Rubisco for HL plants were 31.8, 69.0, and 46.1%, respectively, of the controls. Initial and total FBPase activities were reduced under drought, whereas the activation state of this enzyme was not affected by water stress. By contrast, drought had no significant effect on the activities or activation states of NADP-MDH for all the treatment combinations.

Membrane peroxidation and oxidative stress

Drought obviously reduced the MSI, but increased MDA and H_2O_2 content, especially in HL plants (Fig. 6A–C). Linear analyses were made between MDA content and ion leakage and showed that MDA was positively and significantly correlated with the ion leakage (R=0.93, P <0.01, n=54), indicating that enhanced cell membrane peroxidation may explain most of the increased cell membrane permeability.

To investigate the effects of H_2O_2 and MDA content on Rubisco activity or g_s further, correlation analyses were made between them (Fig. 7A–C). Rubsico initial activity was negatively and significantly correlated with H_2O_2 content (R=0.96, P <0.01, n=18). Meanwhile, there were negative and significant relationships between g_s and H_2O_2 (R=0.95, P <0.01, n=54), and MDA (R=0.97, P <0.01, n=54), respectively.

Discussion

Rice normally suffers no drought stress as a wetland plant grown in paddy fields in the hot summer. If rice is grown aerobically (i.e. as a dry land crop; Bouman *et al*., 2005) or with less irrigation, will its photosynthetic machinery be more sensitive to a combination of drought and high light stresses? In this study, when rice was subjected to different extents of drought and irradiance such that leaf *RWC* was reduced, its capacity to assimilate carbon dioxide was reduced and a clear photoinhibition of photosynthesis was observed under high light. This is a response similarly found in other dryland plants (Björkman and Powles, 1984; Valladares and Pearcy, 1997). The decrease in photosynthetic carbon assimilation found under the drought conditions implies the occurrence of both stomatal and non-stomatal limitation of photosynthesis.

Many authors suggest that diffusive (stomatal and mesophyll) limitations are most important for most drought situations (leaf RWC down to 70–75%) (see Chaves *et al*., 2002, 2003, for reviews). It has been known that if stomatal limitation is exclusively involved in the inhibition of A, exposure to saturating CO_2 concentrations should be effective in restoring A_{max} in previously droughted plants to values comparable with the control. Accordingly, in our experiments, independent of irradiance, as *RWC* falls from *c*. 92% to 79%, stomatal limitation was largely responsible for the inhibition of A, since considerable recovery of A_{max} was observed in previously droughted plants, whereas mesophyll or

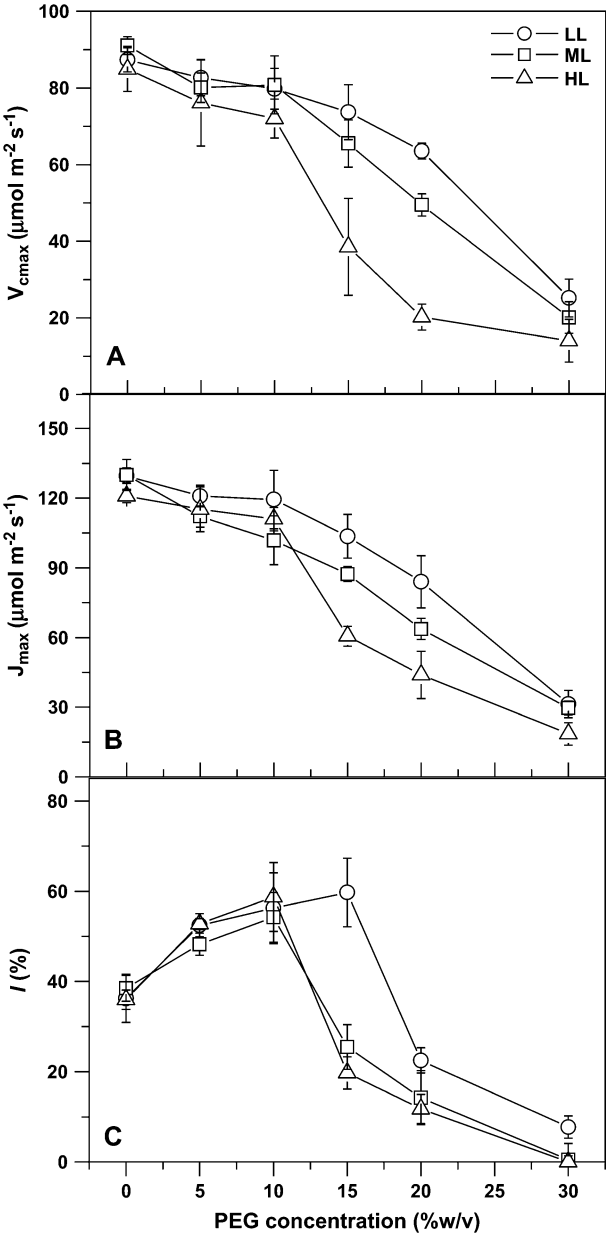

Fig. 3. Effects of 2 d exposure to PEG treatment under low light (LL), medium light (ML), and high light (HL) on maximum velocity of RuBP carboxylation by Rubisco ($V_{cmax,}$ A), maximum potential rate of electron transport contributed to RuBP regeneration ($J_{max,}$ B) and stomatal limitation (*l*, C) in rice leaves. Circles, LL; squares, ML; triangles, HL. Data are the means of four replicates with standard errors shown by vertical bars.

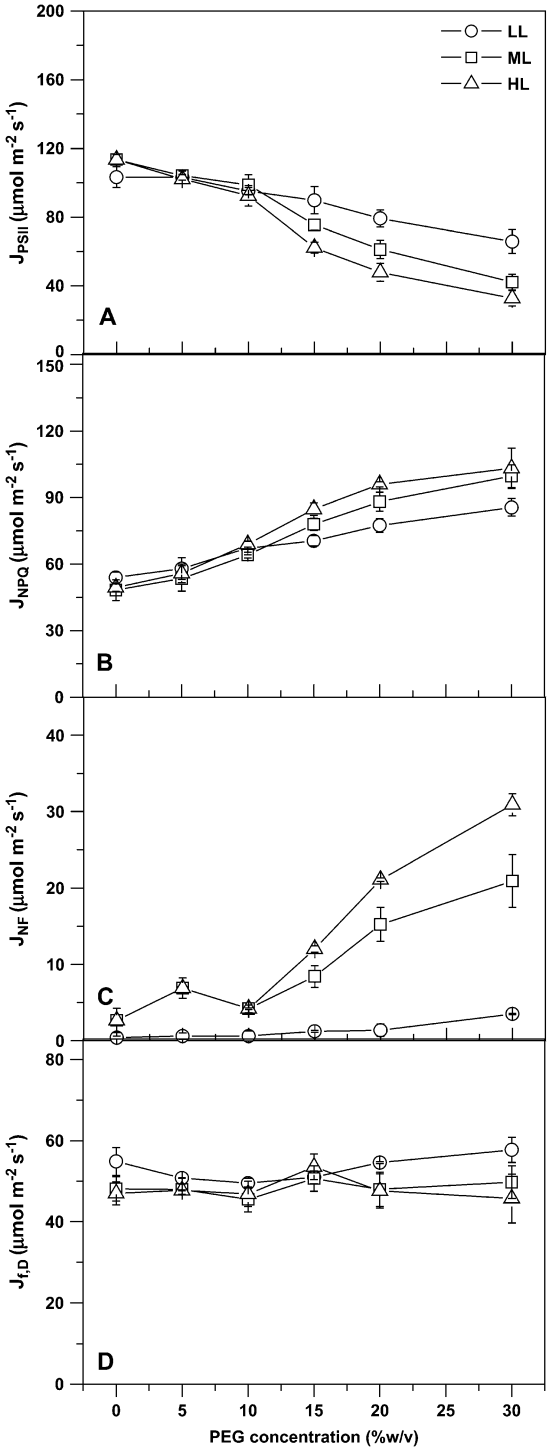

Fig. 4. Effects of 2 d exposure to PEG treatment under low light (LL), medium light (ML), and high light (HL) on the energy flux via linear electron transport (J_{PSII}, A), ΔpH- and xanthophyll-regulated thermal energy dissipation (J_{NPQ}, B), thermal dissipation in non-functional PSII (J_{NF}, C), and fluorescence and constitutive thermal dissipation ($J_{f,D}$, D) in rice leaves. Measurements were made at 25 °C and 500 μmol m^{-2} s^{-1}. Circles, LL; squares, ML; triangles, HL. Data are the means of four replicates with standard errors shown by vertical bars.

metabolic limitation dominated the inhibition when *RWC* is below 79%, since A_{max} showed very poor recovery. However, it is argued that the metabolic limitations may not be true as, in general, drought combined with high CO_2 causes a very low mesophyll conductace as shown by Centritto *et al*. (2003). Accordingly, the methods based on g_i analysis were used to calculate V_{cmax} and J_{max}. These results showed that, as *RWC* was below 70%, the PEG-induced reduction in photosynthetic capacity was

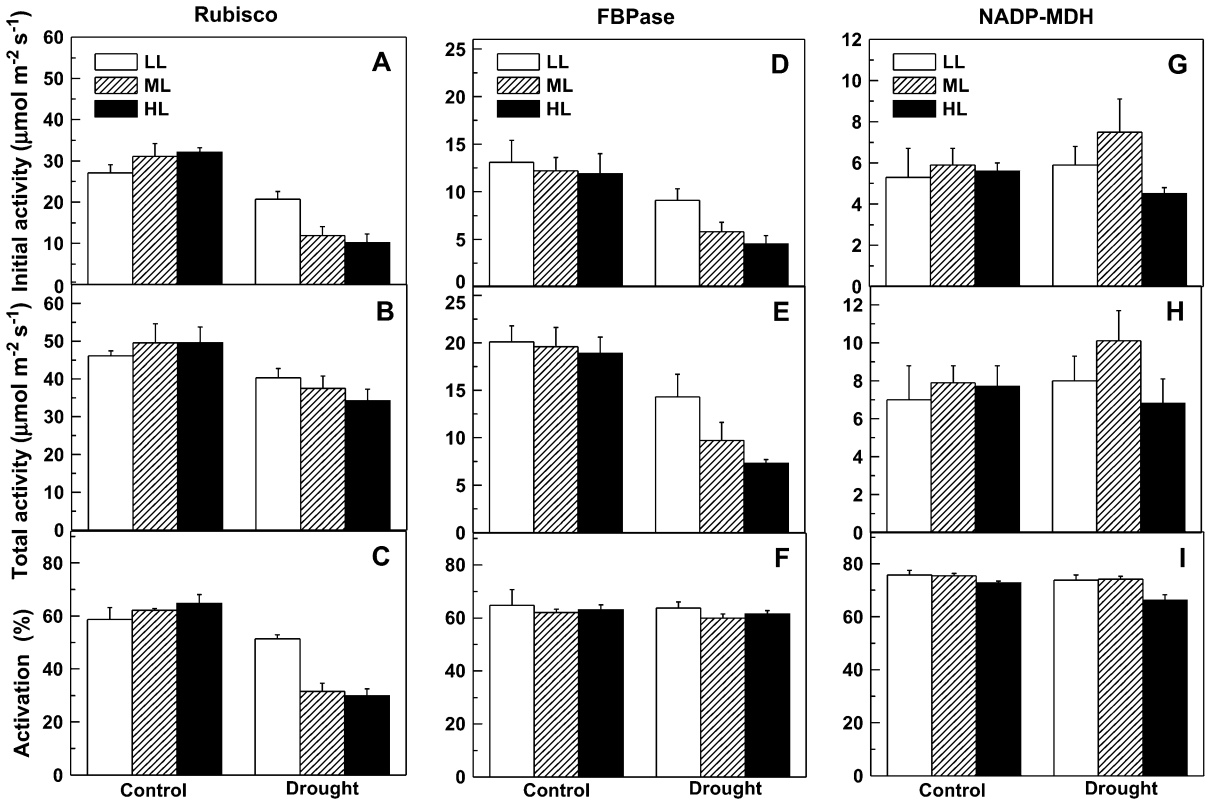

Fig. 5. Effects of PEG treatment under low light (LL), medium light (ML), and high light (HL) on the initial and total activities (μmol m^{-2} s^{-1}) and the activation states of ribulose-1,5-*bis*phosphate carbosylase oxygenase (Rubisco, A–C), stromal fructose-1,6-*bis*phosphatase (FBPase, D–F), and NADP-dependent malate dehydrogenase (NADP-MDH, G–I) in rice plants 2 d after the start of the 30% PEG treatment. White column, LL; striped column, ML; black column, HL. Data are the means of four replicates with standard errors shown by vertical bars.

always accompanied by a decrease in both V_{cmax} and J_{max}. Inactivation or loss of Rubisco would reduce the carboxylation efficiency (V_{cmax}) while the reduction in J_{max} is associated with the diminution of sedoheptulose-1,7-*bis*phosphatase (SBPase) and fructose-1,6-*bis*phosphatase, the key regulatory enzymes in the Calvin cycle (Allen *et al*., 2000; Nogués and Baker, 2000; Ölcer *et al*., 2001). In fact, these results clearly showed that the activity of Rubisco and stromal FBPase, as well as the activation state of Rubisco, were inhibited by 30% PEG which therefore limited photosynthesis (Fig. 5). These results suggest that the enzyme involved in RuBP carboxylation by Rubisco and RuBP regeneration limits photosynthesis under water stress (Gunasekera and Berkowitz, 1993; Sanchez-Rodriguez *et al*., 1997). We therefore conclude that the decline of photosynthesis under severe drought was independent of internal [CO_2], but the activity of the Calvin cycle. However, these responses are not always observed. For example, drought had no effect on Rubisco activity in maize (Castrillo *et al*., 2001). Therefore, it appears that different changes in enzyme will occur in different plants and experimental conditions under water stress, indicating that other metabolic processes (e.g. ATP synthesis) might also be responsible for loss of photosynthetic capacity (Tezara *et al*., 1999).

Plants frequently encounter irradiances that exceed their photosynthetic capacity, especially when their capacity for CO_2 fixation is reduced by stress conditions. Protection mechanisms that prevent the production of excessive reducing power are thus an important strategy under these conditions. Such protection may be achieved by the regulated thermal dissipation occurring in the light-harvesting complex, involving the xanthophyll cycle and presumably the lutein cycle (Müller *et al*., 2001). Interestingly, it was found that a decrease in energy partitioning to the linear electron transport in PSII (J_{PSII}) was due to the stress treatment, accompanied by an increase in inactive PSII (J_{NF}) and light-regulated thermal dissipation in active PSII (J_{NPQ}). However, $J_{f,D}$ maintained about 50 μmol m^{-2} s^{-1}. The redistribution of absorbed light energy should help regulate and protect photosynthesis in drought conditions in which light energy absorption exceeds the capacity for light utilization (Flexas and Medrano, 2002*b*).

When *RWC* fell from *c*. 92% to 79%, the decline in non-cyclic electron transport (as indicated by J_{PSII}; Fig. 4A) was less than the decrease observed in the rate of CO_2 assimilation (Fig. 2A), suggesting an increase in the activity of another sink for the absorbed energy under drought, such as photorespiration (Harbinson *et al*., 1990;

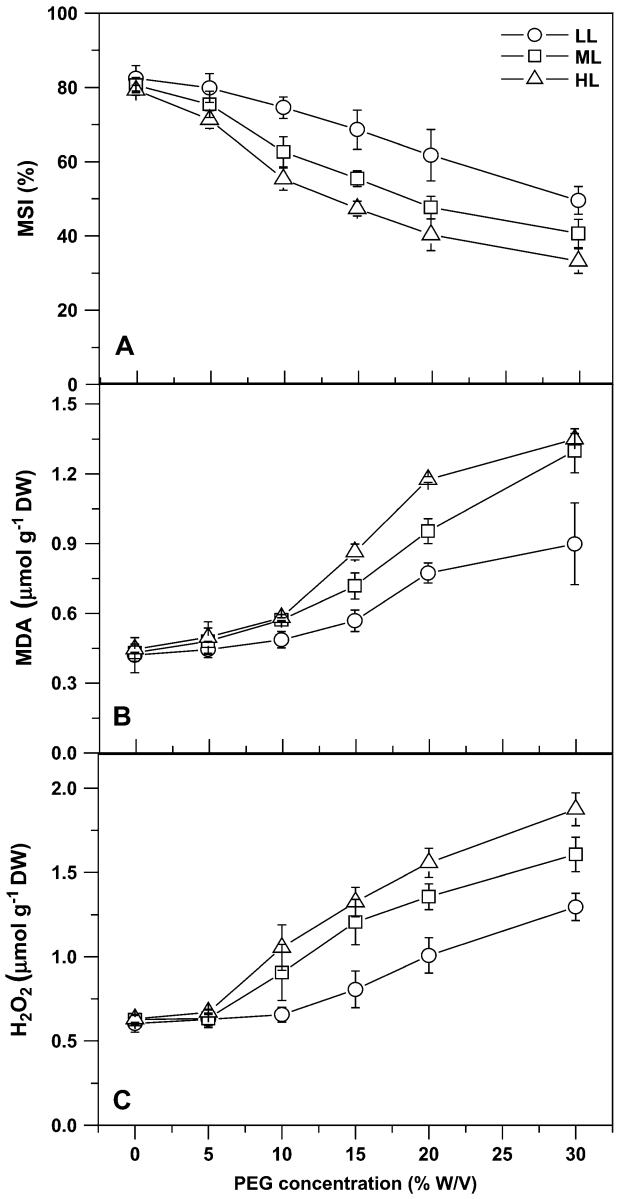

Fig. 6. Effects of 2 d exposure to PEG treatment under low light (LL), medium light (ML), and high light (HL) on the membrane stability index (MSI, A), MDA content (B), and H_2O_2 content (C) in rice leaves. Circles, LL; squares, ML; triangles, HL. Data are the means of four replicates with standard errors shown by vertical bars.

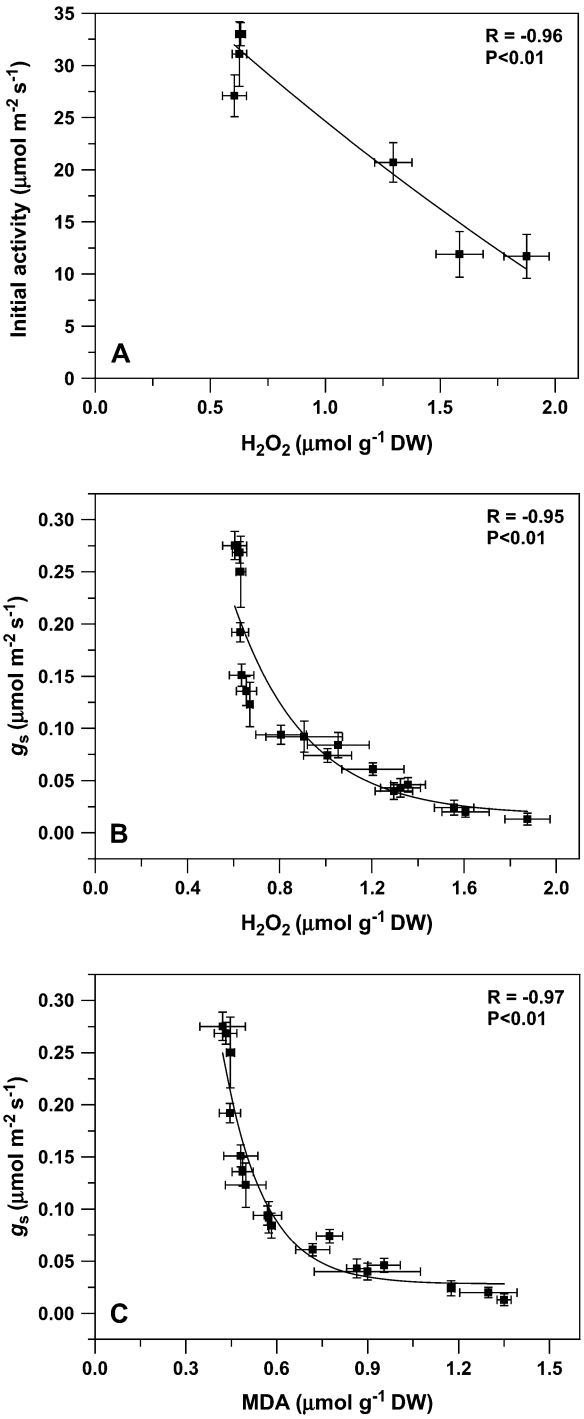

Fig. 7. The relationships between Rubisco initial activity and H_2O_2 content, between g_s and H_2O_2 content, and between g_s and MDA content. Rubisco initial activities were obtained from Fig. 5, H_2O_2 and MDA content were obtained from Fig. 6. Data are the means of four replicates with standard errors shown by vertical bars.

Wingler *et al*., 1999) or Mehler reaction (Biehler and Fock, 1996). This flux of electrons to oxygen may support PSII activity and avoid an over-reduction of the stroma, as suggested by the unaltered activation state of NADP-MDH under drought. Meanwhile, the electron transport to O_2 is an effective way to dissipate excess energy in drought-stressed leaves (Powles and Osmond, 1978; Osmond *et al*., 1980). It is known from many studies that, in droughted leaves, photorespiration is essential and more effective than the Mehler reaction in protecting plants against photodamage under excessive light intensities or water deficit (Wu *et al*., 1991; Heber *et al*., 1996). However, Biehler and Fock (1996) reported that the Mehler reaction was an important sink for reducing power in stressed wheat leaves when photosynthetic electrons were in excess of demand.

When the conjunct operation of all the dissipation mechanisms described above fails to dissipate all the energy absorbed by the leaf safely, the probability of PSII photoinhibition and photoinactivation increases, which leads to more inhibition of light-saturated photosynthesis, photon yield, and electron transport. In fact, the electrons allocated to the inactive photosystem II centres (J_{NF}) increased with the severity of the stresses in our study. Photoinactivation of PSII includes the loss of function of the D1 protein and a D1-repairing mechanism that operates in the chloroplasts and maintains the balance between functional and non-functional PSIIs (Melis, 1999; Flexas *et al*., 2001). The main function of these inactivated centres would be to rebuild PSII and to maintain the remaining PSII function, since inactive centres are capable of non-radiative energy dissipation (Lee *et al*., 2001). Earlier studies suggest that drought stress can cause a considerable depletion of the PSII core, enhancing the degradation of CP43 as well as D1 protein and interfering with the phosphorylation of PSII core proteins (Giardi *et al*., 1996). In this study, an increase in non-functional PSIIs under drought and high light indicated that D1-repairing mechanisms were involved in the dissipation of excess light. However, if a lower amount of D1 protein occurred in drought-stressed leaves, it means that the enhanced synthesis of D1 protein cannot match its degradation (Giardi *et al*., 1996).

Recently, it has been suggested that metabolic responses to severe water stress and high light occur indirectly as a consequence of oxidative stress, rather than as a direct response to water shortage (Flexas *et al*., 2006). Intriguingly, we also found the marked accumulation of H_2O_2 and MDA when g_s decreased from a maximum to about 0.15 mol H_2O m^{-2} s^{-1} indicating that oxidative stress had occurred. H_2O_2 can act as a local or systemic signal for leaf stomata closure and leaf acclimation to high irradiance (Karpinska *et al*., 2000). However, when the production of H_2O_2 exceeds a threshold, programmed cell death will occur (Houot *et al*., 2001). Upon the accumulation in reactive oxygen species, an extensive peroxidation and de-esterification of membrane lipids, as well as protein denaturation and DNA mutation accordingly occurred (Bowler *et al*., 1992). Actually, a combined effect between drought and high irradiance on cell membrane integrity (MSI) in this study was more profound than a single factor alone (Fig. 6A). Lipid peroxidation inevitably results in cell ultrastructural trauma, which takes a prominent role in drought-induced cell death. In the present experiment, severe drought with high light led to an increase in ion leakage and lipid peroxidation with the decreases in stomatal conductance (Fig. 6). The close correlations between H_2O_2 and g_s, and MDA and g_s, further demonstrated the adverse effects on stomatal characteristics by H_2O_2 accumulation and lipid peroxidation (Fig. 7). Furthermore, a close negative correlation was also observed between H_2O_2 content and initial Rubisco activity (R=0.96, P <0.01, n=18) for the 30% PEG-treated plant (Fig. 7A). H_2O_2 has detrimental effects on Rubisco (Ishida *et al*., 1998) and the redox state it regulates also affects the expression of chloroplast and nuclear genes (Irihimovitch and Shapira, 2000; Pfannschmidt, 2003). Accordingly, the decreases in Rubisco activity under severe drought with high light are likely to be related to the H_2O_2 accumulation and the associated changes in the redox state, which strongly supports the idea that the depressed photosynthetic metabolism is a result of oxidative stress occurring in severely stressed plants in which antioxidant capacity is not enough to cope with the generation of reactive oxygen species (Flexas *et al*., 2006). More study should be conducted to follow this up.

In conclusion, it has been demonstrated that, as a wetland plant, rice photosynthesis is very sensitive to the leaf water status, as measured by loss of *RWC* and water potential. The drought-induced inhibition of photosynthesis under different irradiance levels in this study was due to both diffusive and metabolic limitation: diffusive limitations that occurred due to stomatal closure and low mesophyll conductance to CO_2 upon moderate drought conditions; and metabolic limitations that generally occurred upon more severe water stresses. Metabolic limitation of photosynthesis may be related to the adverse effects of some metabolic processes and the oxidative damage to the chloroplast. Meanwhile, an enhanced thermal dissipation is an important process to minimize the adverse effects of drought and high irradiance when CO_2 assimilation is suppressed.

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