PHOTOSYNTHETICA 43 (4): 501-508, 2005

Photoprotective effects of high level expression of C_4 phospho*enol*pyruvate carboxylase in transgenic rice during photoinhibition

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Abstract

With untransformed rice cv. Kitaake as control, the characteristics of carbon assimilation and photoprotection of a transgenic rice line over-expressing maize phospho*enol*pyruvate carboxylase (PEPC) were investigated. The PEPC activity in untransformed rice was low, but the activity was stimulated under high irradiance or photoinhibitory condition. PEPC in untransformed rice contributed by about 5–10 % to photosynthesis, as shown by the application of the specific inhibitor 3,3-dichloro-2-(dihydroxyphosphinoylmethyl)propenoate (DCDP). When maize PEPC gene was introduced into rice, transgenic rice expressed high amount of maize PEPC protein and had high PEPC activity. Simultaneously, the activity of carbonic anhydrase (CA) transporting CO_2 increased significantly. Thus the photosynthetic capacity increased greatly (50 %) under high CO_2 supply. In CO_2-free air, CO_2 release in the leaf was less. In addition, PEPC transgenic rice was more tolerant to photoinhibition. Treating by NaF, an inhibitor of phosphatase, showed that in transgenic rice more phosphorylated light-harvesting chlorophyll *a/b*-binding complexes (LHC) moved to photosystem 1 (PS1) protecting thus PS2 from photo-damage. Simultaneously, the introduction of maize PEPC gene could activate or induce activities of the key enzymes scavenging active oxygen, such as superoxide dismutase (SOD) and peroxidase (POD). Hence higher PS2 photochemical efficiency and lower superoxygen anion ($O_2^{\cdot-}$) generation and malonyldiadehyde (MDA) content under photoinhibition could improve protection from photo-oxidation.

Additional key words: carbon assimilation; carbonic anhydrase; chlorophyll fluorescence; 3,3-dichloro-2-(dihydroxyphosphinoylmethyl)propenoate; enzymes scavenging active oxygen; malonyldialdehyde; *Oryza*; peroxidase; superoxide dismutase.

Introduction

Phospho*enol*pyruvate carboxylase (PEPC) is an oligomeric, cytosolic enzyme found ubiquitously and widely distributed in different parts or organs of all plants (O'Leary 1982, Chollet *et al*. 1996). Its numerous physiological functions within the plant include plant host-cell C_4-acid formation in N_2-fixing legume root nodules (Deroche and Carrayol 1988), stomatal opening in leaf guard cells (Outlow 1990), C/N partition in C_3 plants, seed formation and germination, and fruit ripening (Lepiniec *et al*. 1994). In particular, PEPC functions in the CO_2 concentration mechanism of C_4 plants, being a key enzyme for the fixation of atmospheric CO_2 during C_4 and CAM photosynthesis. However, PEPC function has not been widely studied in C_3 plants, due to its low endogenous activity. Multiple enzymes in the C_4 photosynthetic pathway exist in leaves of wheat and other C_3 crops (Aoyagi and Bassham 1985, 1986). Indeed, use of a PEPC inhibitor showed that PEPC contributes by more than 10 % to photosynthesis (Jenkins 1989). Later developments in molecular biology techniques revealed that the PEPC genes and proteins had great homology in C_3 and C_4 plants. Introduction of C_4 PEPC genes into C_3 plants such as tobacco (Dong *et al*. 1995), potato (Gehlen *et al*. 1996), and rice (Fukayama *et al*. 2003) did not

Received 16 November 2004, *accepted* 27 January 2005.
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Abbreviations: CA – carbonic anhydrase; Chl – chlorophyll; DCDP – 3,3-dichloro-2-(dihydroxyphosphinoylmethyl)propenoate; F_v/F_m – PS2 photochemical efficiency; LHC – light-harvesting chlorophyll *a/b*-binding complexes; MDA – malonyldialdehyde; NADP-MDH – NADP-malate dehydrogenase; NADP-ME – NADP-malic enzyme; $O_2^{\cdot-}$ – superoxygen anion; PEPC – phospho*enol*pyruvate carboxylase; POD – peroxidase; PPDK – pyruvate orthophosphophate dikinase; PS – photosystem; PPFD – photosynthetic photon flux density; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase; SOD – superoxide dismutase.
Acknowledgement: This work was supported by the National Key Basic Research and Development Plan of China (G1998010100).

result in high photosynthesis activity, but Ku *et al*. (1999) expressed high level of the C_4 maize PEPC gene in rice and observed PEPC activity exceeding that in maize. At present, the effects of various stresses on photosynthetic activities of newly-developed super-high-yield rice hybrids are intensively studied (Chen *et al*. 2004).

Through systematic selection and identification in natural conditions we obtained the 7th generation of stable rice germplasms showing high photosynthetic capacity under strong irradiance and high tolerance to photooxidation (Jiao *et al*. 2001, 2002). Traditionally, there have been different views about the function of C_4 photosynthetic enzymes in the leaves of C_3 plants. Previous reports suggested that the C_4 enzymes functioned not as photosynthetic enzymes, but rather were induced by stresses such as wounding, low oxygen, low temperature, and salinity, as shown in rice (Fushimi *et al*. 1994) and bean (Walter and Grimma-Pettenati 1994). However, PEPC activity in rice was induced under oxidative stress (Jiao and Ji 1996), perhaps to compensate for stress-induced photo-damage. Some researchers have proposed the presence of a complete C_4 photosynthetic enzymes system: PEPC, pyruvate orthophosphophate dikinase (PPDK), NADP-malic enzyme (NADP-ME), and NADP-malate dehydrogenase (NADP-MDH)) functioning as a limiting C_4 cycle in the leaves of C_3 plants (Li *et al*. 2000, Jiao *et al*. 2001). The introduction of PPDK into tomato changed the plant carbon metabolism and strengthened its limited C_4 photosynthetic pathway (Imaizumi *et al.* 1997). Comparison of PEPC transgenic rice with untransformed rice showed similar photosynthetic rates under PPFD of 200–1 000 μmol(photon) m^{-2} s^{-1}, but under irradiances of 1 200–1 400 μmol(photon) m^{-2} s^{-1} the photosynthetic rate of PEPC transgenic rice increased by 50 % (Jiao *et al*. 2002). A fairly large amount of $^{14}CO_2$ was bound in aspartate in the PEPC transgenic rice (Jiao *et al.* 2003). In order to elucidate the photo-protective effects of PEPC in the leaves of C_3 plants, we used untransformed rice Kitaake and PEPC transgenic rice to compare the adaptive regulation and physiological function of PEPC under adverse irradiances.

Materials and methods

Plants: *Japonica* rice (*Oryza sativa*) Kitaake and PEPC transgenic rice (7th generation, Jiao *et al*. 2001, derived from 3rd generation, Ku *et al*. 1999) was grown in 4 000 cm^3 pots and maintained in a naturally irradiated net house at Jiangsu Academy of Agricultural Sciences, Nanjing, China. There were 5 hills per pot and 1 seedling per hill. Plants were watered and fertilized conventionally.

Photoinhibitory treatment under low CO_2 and O_2: At the heading stage, attached leaves were placed in a leaf chamber and flushed with N_2 (including 1 % O_2) for 3 h under a photosynthetic photon flux density (PPFD) of 1 000 μmol(photon) m^{-2} s^{-1}, supplied by a tungsten-halogen lamp. These conditions (low CO_2 and O_2) act according to Powles (1984) as a photooxidation treatment. Control leaves were treated with a PPFD of 1 000 μmol(photon) m^{-2} s^{-1} and supplied with air.

Photosynthetic O_2 evolution: Leaves under high irradiance of 1 000 μmol(photon) m^{-2} s^{-1} in air or under photoinhibitory condition [1 000 μmol(photon) m^{-2} s^{-1}, low O_2 and CO_2-free atmosphere] were harvested. Leaf segments of 1 cm^2 were cut and put into solution of 100 mM Hepes-KOH (pH 7.8) and 20 mM $NaHCO_3$. The rate of O_2 evolution was measured using an *SP-2* oxygen electrode (*Sinica Academy*, Shanghai, China) under PPFD of 800 μmol(photon) m^{-2} s^{-1} at 28 °C.

Exchange of CO_2: At the heading stage, attached leaves were placed in a leaf chamber, N_2 flushed, at a PPFD of 1 000 μmol(photon) m^{-2} s^{-1} and 28 °C. The rate of CO_2 evolution was measured using a CO_2 infrared gas analyzer (*S-225*, Hoddesdon, UK).

The control and photoinhibited leaves were collected and kept in liquid N_2 for enzyme analyses.

PEPC activity: According to the methods of Gonzalez *et al*. (1984) and Ku *et al*. (1999), about 0.25 g of leaf tissue was harvested from newly mature leaves from each plant in the light and quickly ground in 1.5 cm^3 extraction buffer containing 50 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 5 mM dithiothreitol (DTT), 2 % (m/v) insoluble polyvinylpolypyrrolidone (PVP), and 10 % glycerol. After total maceration, the crude extract was centrifuged at 13 000×*g* for 10 min at 4 °C. PEPC was immediately assayed spectrophotometrically at room temperature in a mixture containing 50 mM Hepes-KOH, pH 8.0, 10 mM $NaHCO_3$, 5 mM $MgCl_2$, 1.5 units of malate dehydrogenase (MDH), 0.2 mM NADH, and 20–50 mm^3 of enzyme extract. Adding PEP to a final concentration of 2 mM started the reaction. The change in NADH was monitored by a spectrophotometer at 340 nm.

Carbonic anhydrase (CA) activity: According to Guo *et al*. (1988), about 5 g of leaf tissue was harvested from mature leaves of several plants in the light and quickly ground in 30 cm^3 of extraction medium (5 mM DTT, 10 mM barbital buffer, pH 8.2) using pre-chilled mortar and pestle. The homogenate was filtered through eight layers of miracloth and centrifuged at 5 000×*g* for 5 min. The supernatant was used for CA assay in a vessel after injection with pure CO_2 during few minutes. The activity of CA was calculated from the rate of change in pH of the reaction solution.

Superoxide dismutase (SOD) activity: According to the method of Giannopolitis and Ries (1977), 0.2 g of blades were thoroughly ground in a mortar and homogenized in an ice bath until no fibrous residue could be seen. The grinding medium [4 000–6 000 cm^3 kg^{-1}(FM)] consisted of 0.1 M K_2HPO_4 and 0.1 mM EDTA (pH 7.8) plus homogenizing glass beads and PVP in ice-bath. The homogenate was centrifuged at 10 500×*g* for 20 min in a *Sorvall RC2-B* refrigerated centrifuge at 4 °C. The supernatant was referred to as the crude SOD extract. The reagent [adding 2 cm^3 3 μM EDTA made up with 50 mM of a pH 7.8 phosphate buffer, 2 cm^3 of 2.25 mM nitroblue tetrazolium (NBT) and 2 cm^3 of 1 μM hepatoflavin to 54 cm^3 of 14.5 mM methionine in the dark] mixed with proper SOD crude enzyme in transparent tube shelf was irradiated for 10 min at PPFD of 2 000 μmol(photon) m^{-2} s^{-1} in the cultivation box. After stopping the reaction, the change at 560 nm was determined. SOD activity was assessed by its ability to inhibit the reduction of NBT by the $O_2^{\cdot-}$ generation system xanthine oxidase producing an increase in absorbance at 560 nm of about 0.02 units per min at 25 °C in the absence of enzyme. One unit of SOD activity is defined as the amount of enzyme that causes 50 % inhibition of initial rate of NBT reduction.

Peroxidase (POD) activity: According to Kochba *et al.* (1992), 0.2 g blades were put in pre-cold mortar and homogenized in 1 cm^3 of 50 mM (pH 7.8) phosphate buffer. The homogenate was centrifuged at 10 500×*g* for 20 min under 4 °C. The supernatant was crude enzyme. 3 cm^3 of the reagent [100 cm^3 of 50 mM phosphate buffer (pH 7.8) with 28 mm^3 guaiacol and 19 mm^3 of 30 % H_2O_2] was mixed with 10 mm^3 of crude enzyme and the change of optical density (OD) was recorded at 470 nm.

The chlorophyll (Chl) fluorescence parameters were measured using an *FMS-2* fluorescence meter (*Hanstach*, UK) and calculated according to Genty *et al.* (1989). The rice leaves were dark-adapted for 30 min and then irradiated by weak modulated measuring beam [0.12 μmol(photon) m^{-2} s^{-1}] to determine the initial fluorescence yield (F_0). Maximum fluorescence yield (F_m) was determined during a saturating photon pulse [4 000 μmol(photon) m^{-2} s^{-1}]. Variable Chl fluorescence (F_v) was calculated as $F_v = F_m - F_0$. Primary PS2 photochemical efficiency was expressed as F_v/F_m.

Measurement of F_{685}/F_{735} special ratio: PEPC transgenic rice and Kitaake were divided into two groups, one group placed in the dark, another group put under high irradiance [1 000 μmol(photon) m^{-2} s^{-1}] for 3 h. Then chloroplasts were extracted. Measurements of low temperature fluorescence at 77 K were done with a *44W*-fluorescence spectrofluorimeter. The excitation beam of 440 nm provided by a 75 W tungsten lamp and a 440 nm filter was focused on the thylakoid sample held at 77 K in a quadratic glass cup. The fluorescence signals were collected with a diode array detector, and the fluorescence emission spectrum between 650 and 750 nm was recorded by a dual-channel potentiometric recorder. F_{685}/F_{735} was determined by low temperature (77 K) steady-state and room temperature fluorescence spectroscopy.

$O_2^{\cdot-}$ generation rate: According to the method of Wang and Luo (1990), leaf segments (about 0.5 g fresh mass) were homogenized using a chilled pestle and mortar with acid-washed quartz sand in 65 mM phosphate buffer (pH 7.8). The homogenate was filtered through 4 layers of *Miracloth*. The filtrate was centrifuged at 2 500×*g* for 10 min at 4 °C. Phosphate buffer (0.9 cm^3) and 0.1 cm^3 of hydroxylamine (10 mM) were added to 1 cm^3 of supernatant. The mixture reacted at 25 °C for 20 min, 1 cm^3 of the reacting mixture was injected into 1 cm^3 of 17 mM *p*-aminobenzene sulfonic acid and 1 cm^3 of 17 mM α-naphthylamine at 25 °C for 20 min. The above mixture was extracted by ether. Absorbance of water phase was measured at 530 nm. The standardization curve of NO_2^- was produced by a similar procedure. $O_2^{\cdot-}$ generation rate = $O_2^{\cdot-}$ production/reaction time × the amount of protein [mmol(O_2) kg^{-1}(protein) s^{-1}].

Malonyldiadehyde (MDA): In the method of Heath and Packer (1968), the reaction between MDA (1 mol) and thiobarbituric acid (TBA, 2 mol) forms red-blow trimethine. Leaf blades (0.5 g) were ground in a solution containing 5 cm^3 of 10 % trichloroacetic acid (TCA) and a small amount of quartz sand. The homogenate was centrifuged for 10 min at 3 000×*g*. The supernatant (2 cm^3) was further mixed with 2 cm^3 of 0.67 % TBA (m/v). After keeping in boiling water for 20 min and cooling, the mixture was again centrifuged for 10 min at 3 000×*g*. The supernatant was measured at 532 and 600 nm with a spectrophotometer. The content of MDA was calculated by the following equation: $\varepsilon_{532\text{-}600nm} = 1.55\times10^5\ M^{-1}\ cm^{-1}$.

Western blot analysis: Rice leaves (0.5 g fresh mass) were harvested and ground in extraction medium [50 mM Tris-HCl (pH 7.5) containing 1 mM $MgCl_2$, 5 mM DTT, and 2 % (m/v) insoluble PVP]. After complete maceration, the crude extract was centrifuged at 13 000×*g* for 10 min, and proteins were sampled per well at room temperature and constant voltage (80 V) for 2 h. The gel was soaked in transfer buffer (150 mM glycine, 20 mM Tris-HCl, 0.01 % SDS, 4 % methanol, pH 8.8). Then a nitrocellulose membrane (NC) was applied and the set-up was electrophoresed at 250 mA for 1.5 h, in order to transfer the protein onto the NC. The transferred NC was soaked in blocking buffer [1 % non-fat milk powder, 20 mM Tris-HCl (pH 8.0), 100 mM NaCl] at room temperature for 2 h before addition of the antibody (anti-PEPC, -CA, RuBPCO LSU or -RuBPCO SSU) and incubated at room temperature for 1 h. Following this incubation, the NC was washed by PBS (3×, 10 min each), and goat anti-

rabbit Ig G (1 : 1 500) was added into blocking buffer for 1 h. Then the NC was washed with TBS (20 mM Tris-HCl, pH 8.0, 100 mM NaCl; 3× for 10 min each). Finally, the NC was developed using AP (100 mM Tris, 100 mM NaCl, 5 mM $MgCl_2$, BCIP/NBT).

Results

Changes of PEPC activity and CA activity in PEPC transgenic rice and untransformed rice during photoinhibition: PEPC activity in transgenic rice was higher than that in untransformed rice both before and after photoinhibition treatment (Fig. 1*A*). At 0 h [when the sample was collected from flushed air under a PPFD of 600–800 µmol(photon) m^{-2} s^{-1} at 28 °C], the PEPC activity was 167 and 220 mmol kg^{-1} (Chl) s^{-1} in transgenic and untransformed rice, respectively. After 4 h of artificial photoinhibitory treatment with low CO_2 and high irradiance (Fig. 1*A*; 4 h), the PEPC activity reached 333–361 mmol kg^{-1}(Chl) s^{-1} in transgenic rice and 56 mmol kg^{-1}(Chl) s^{-1} in untransformed rice. This indicates that PEPC activity was induced in both rice genotypes under the adverse irradiances. The PEPC activity in PEPC transgenic rice increased more rapidly and remained uniformly higher than that in the untransformed rice. Western blotting of PEPC protein contents showed that the change in PEPC activity was consistent with the content of PEPC protein. When CA activity and protein content were tested, a similar result was obtained (Fig. 1*B*). After photo-inhibitory treatment for 4 h, the activity of CA in PEPC transgenic rice was six-fold higher than that in the untransformed rice. Together, these results indicate that under photoinhibitory conditions, PEPC transgenic rice showed increased protein contents and activities of enzymes involved in the transport and fixation of CO_2, such as PEPC and CA.

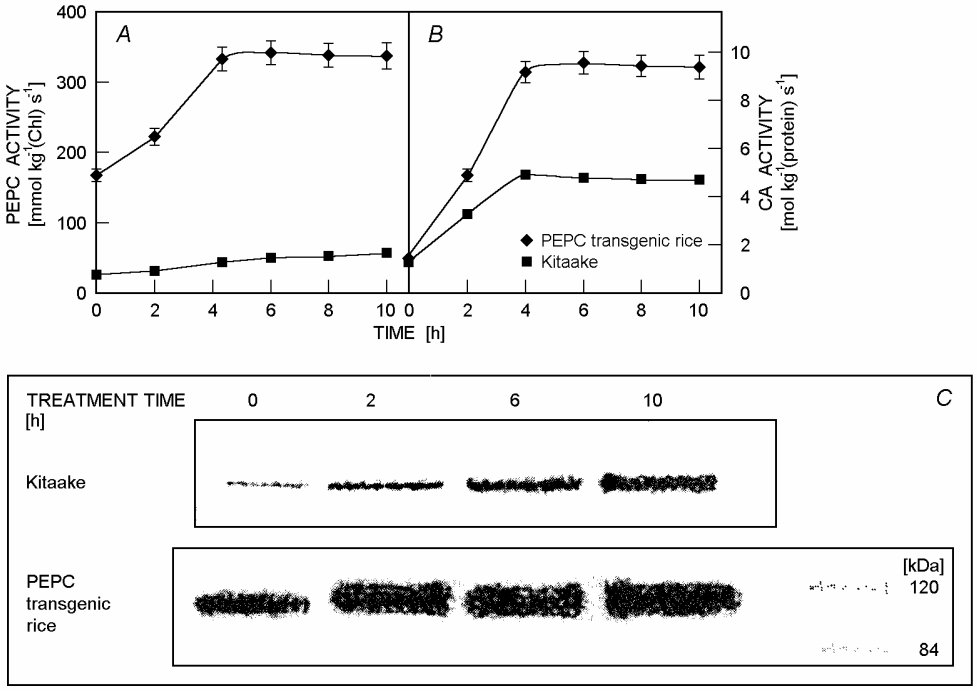

Fig. 1. Changes of phospho*enol*pyruvate carboxylase, PEPC (*A*) and carbonic anhydrase, CA (*B*) activities and PEPC protein amount (Western blot) (*C*) in Kitaake and PEPC transgenic rice during photoinhibition. Means±S.E. expressed as a *bar*. $n = 4$.

CO_2 exchange in PEPC transgenic rice under photoinhibitory conditions: To ascertain the photosynthetic effect of PEPC activity increase induced by the irradiance stress, leaf discs were treated with the PEPC-specific inhibitor, 3,3-dichloro-2-(dihydroxyphosphinoylmethyl)propenoate (DCDP) under the photoinhibitory condition. The rate of O_2 generation in PEPC transgenic rice decreased under these conditions, whereas it decreased much less in untransformed rice (Fig. 2*A*). Hence under photoinhibition, the increased PEPC activity in rice plants expressing the maize PEPC gene helped maintain a higher CO_2 assimilation capacity and stabilize photosynthesis.

To determine the difference in CO_2 release (respiration) and CO_2 re-fixation without photosynthesis in different genotypes, attached rice leaves were placed in a photosynthetic chamber and treated with N_2 flushing and PPFD of 1 000 mol(photon) m^{-2} s^{-1}. Under these conditions, the release of CO_2 reached its highest value at 40 min and remained stable thereafter in both genotypes.

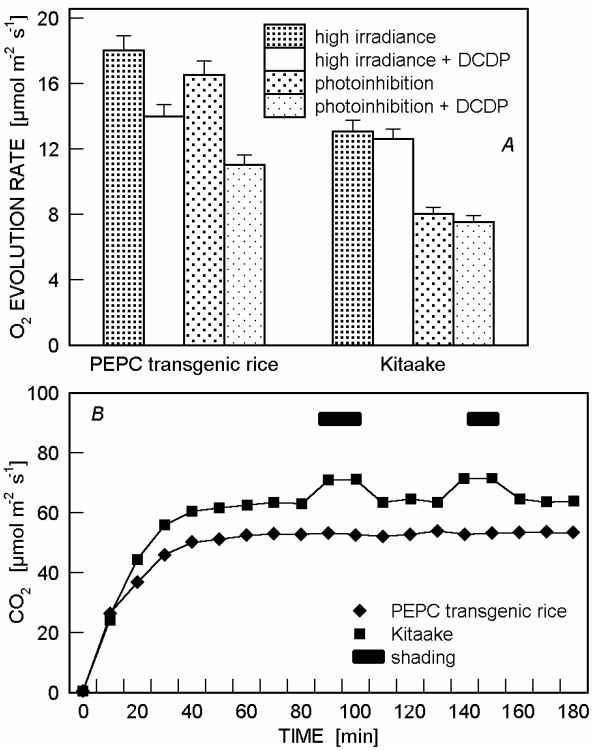

Fig. 2. (*A*): The effect of 3,3-dichloro-2-(dihydroxyphosphinoylmethyl)propenoate (DCDP) on photosynthetic O_2 evolution in Kitaake and PEPC transgenic rice under high irradiance and photoinhibition. Means±SD from three replicates at high irradiance of 1 400 μmol(photon) m^{-2} s^{-1}; photoinhibitory condition: CO_2-free, 2.5 % O_2, PPFD 1 000 μmol(photon) m^{-2} s^{-1}. Significance between PEPC transgenic plant and Kitaake was expressed at 0.01 probability when the two genotypes were treated. Means±S.E. expressed as *bars*. $n = 4$. (*B*): CO_2 exchange in Kitaake and PEPC transgenic rice under PPFD of 1 000 μmol(photon) m^{-2} s^{-1} and CO_2-free condition.

However, the release of CO_2 in PEPC transgenic rice was by 10 μmol m^{-2} s^{-1} lower than that in untransformed rice. Shading of the plants increased CO_2 release in both genotypes, indicating that a small CO_2 re-fixation occurred in the irradiated leaves. Thus PEPC transgenic rice had a higher capacity for CO_2 re-fixation than the untransformed rice. Overall, rice plants expressing the maize PEPC gene showed a larger CO_2 assimilatory capacity and a larger capacity for CO_2 re-fixation under low CO_2 concentrations, indicating that PEPC transgenic rice had a higher tolerance to photoinhibition.

Photoinhibition characteristics in PEPC transgenic rice: The PS2 photochemical efficiency (F_v/F_m) decreased differently in the two genotypes under high irradiance [1 500 μmol(photon) m^{-2} s^{-1}] photoinhibition (Fig. 3*A*). However, the photochemical efficiency decreased less in PEPC transgenic rice, indicating that PEPC transgenic rice was more tolerant to photoinhibition. This was further shown in the ratio of F_{685}/F_{735} (Fig. 3*C*),which represents state transfer from PS2 to PS1 by the changes of ratio of fluorescence at 685 and 735 nm, respectively (El Bissati *et al.* 2000). The F_{685}/F_{735} ratio in PEPC transgenic rice dropped less under strong irradiance (Fig. 3*C*), indicating that its PS2 was less inhibited. Photoinhibition by strong irradiance was completely abrogated by dark treatment, indicating that this is a dynamic and reversible change (Fig. 4*B*). We hypothesize that in this case LHC might be photophosphorylated and separated from PS2, allowing PS2 to avoid photodamage from excessive photons. The disintegrated LHC could then return to PS2 in the dark. To test this conjecture, we treated the experimental system with NaF, a specific inhibitor of phosphatase. This treatment blocked both transgenic and untransformed rice from complete recovery of PS2 (Fig. 4*B*).

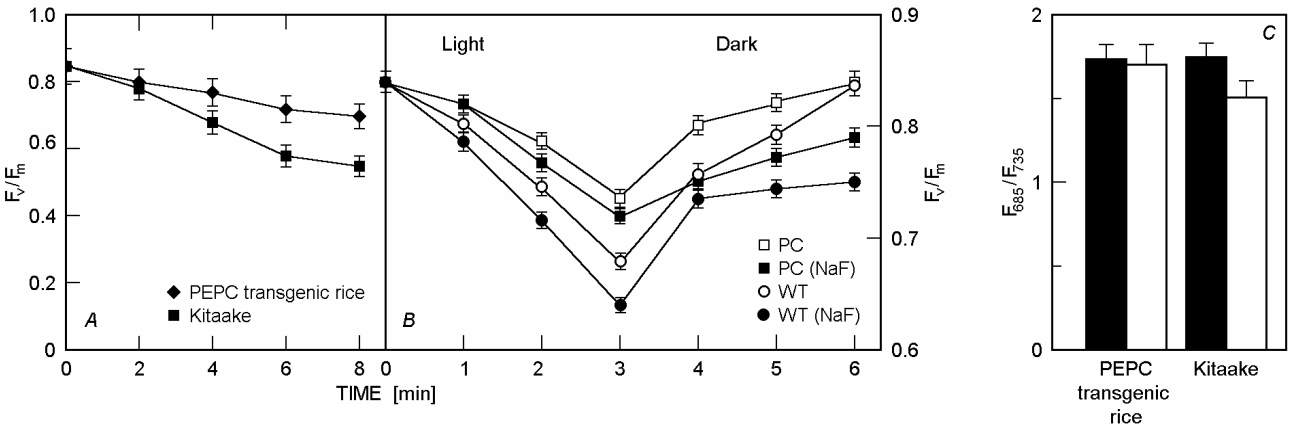

Fig. 3. Changes of F_v/F_m under high irradiance (*A*) or NaF treatment and during recovery (*B*), and (*C*) change of F_{685}/F_{735} in the dark or under high irradiance (3 h) in Kitaake and PEPC transgenic rice. PPFD of 1 500 μmol(photon) m^{-2} s^{-1}, 26 °C. The significance between PEPC transgenic plant and Kitaake for the photophosphorylated dependence is expressed at 0.01 probability when the two genotypes were treated under high irradiance for 3 h and dark for 3 h, respectively (total treatment of 6 h). Means±S.E. expressed as *bars*. $n = 4$.

The recovery in untransformed rice was less efficient than that in PEPC transgenic rice, indicating that in untransformed rice more of phosphorylated LHC moved to PS1 to protect PS2 from photodamage. In the PEPC transgenic rice, PS2 damage was less severe, and the LHC of PS2 was phosphorylated less; thus, NaF was less inhibitory and the change in F_{685}/F_{735} was not severe.

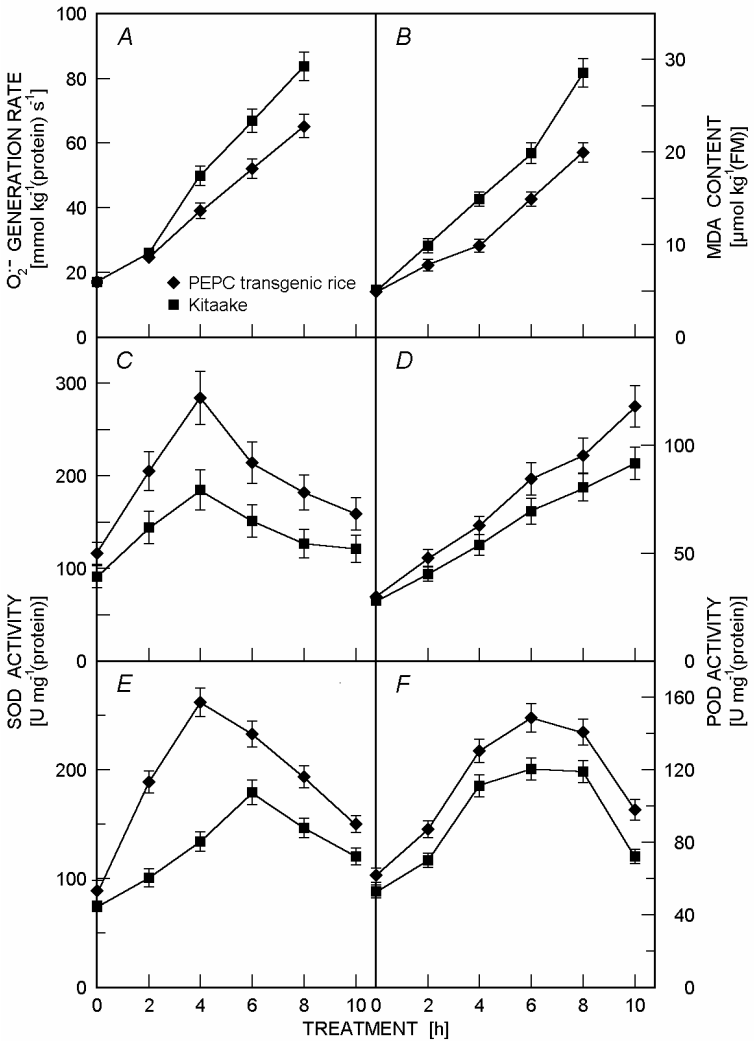

Fig. 4. Changes of (*C, E*) superoxide dismutase (SOD) and (*D, F*) peroxidase (POD) activities and (*A*) $O_2^{\cdot-}$ generation rate and (*B*) malonyldialdehyde (MDA) content in PEPC transgenic rice and Kitaake during photooxidation. Means±S.E. expressed as *bars*. $n = 4$.

Photooxidative characteristics in PEPC transgenic rice: The $O_2^{\cdot-}$ generation rate and membrane peroxidation (MDA content) in PEPC transgenic rice was lower than in untransformed rice under high PPFD and low CO_2 concentration (Fig. 4*A,B*). Hence the photo-oxidative tolerance was higher in PEPC transgenic rice, where the introduced maize PEPC gene might enhance the oxygen scavenging system. In both genotypes under photooxidative treatment, the SOD activity peaked at 4 h and then dropped gradually, while the POD activity increased gradually with time of treatment (Fig. 4*C,D*). These observations suggest that SOD initially actively scavenged oxygen when O_2 was transformed to H_2O_2; thereafter, POD acted as the scavenger. The activities of SOD and POD in the leaves of PEPC transgenic rice were higher than in untransformed rice. Similar results were obtained when PEPC transgenic and untransformed rice plants were subjected to other photooxidation treatments, *e.g.* methyl viologen (MV). Summing up, the introduction of the maize PEPC gene into rice increases activity not only of the gene encoding CA, but also those encoding the photooxidative enzymes SOD and POD.

Discussion

We showed that the activities of PEPC and CA (which is related to the CO_2 concentrating mechanism) were substantially increased under high irradiance and photoinhibition (Fig. 1). Under these conditions, inorganic carbon is converted into C_4 primary photosynthates to act as a CO_2 donor to RuBPCO, allowing the transgenic plants to fix and assimilate more CO_2 (Fig. 2*A*). In the absence of CO_2, a small percentage of CO_2 was released in the photosynthetic cell and re-fixed by a certain minimal rate of photosynthetic carbon metabolism (Fig. 2*B*). The results of ^{13}C analysis (Ku *et al.* 2000) showed that the values of PEPC transgenic rice were similar to those of untransformed rice, though they were somewhat lower. The lower ^{13}C content in the leaves of transgenic plants could be due to fixation of photorespiratory CO_2 by PEPC and RuBPCO. Our results indicated that over-expression of the maize PEPC gene in rice allowed activation of photosynthetic activity or a limiting C_4 cycle under photoinhibition. Under these conditions, more ATP and NADPH would be consumed so as to decrease impediment to energy conversion, resulting in a smaller decrease of F_v/F_m (Fig. 3*A*) and less accumulation of O_2 produced by Mehler reaction and MDA produced by membrane lipid peroxidation (Fig. 4*A*,*B*). High PEPC expression in rice improved CO_2 assimilation under strong or adverse irradiance, thus alleviating photoinhibition and increasing photosynthetic adaptation. On the other hand, our data also showed less photoinhibitory and photooxidative damages in PEPC transgenic rice, and higher activities of active oxygen scavenging enzymes such as POD and SOD in the PEPC transgenic rice (Fig. 4*C*,*D*). More SOD is induced when plants form more $O_2^{\cdot -}$ (Salin 1988). However, our experiments showed that at both photooxidation treatments, PEPC transgenic rice plants had higher SOD and POD activities than untransformed rice did (Fig. 4*C*–*F*). This is consistent with a previous report that introduction of a key gene into transgenic plants could induce expression of parallel expression genes (Gupta *et al*. 1993). We showed that the PEPC transgenic rice had increased PEPC and CA activities, increased CO_2 assimilation, increased consumption of ATP and NADPH, and decreased photoinhibition. In addition, SOD and POD activities increased, leading to decreased accumulation of active oxygen. On the whole, our data show that introduction of the maize PEPC gene into rice produces a variety of photo-protective effects.

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