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Cultivar differences in photosynthetic tolerance to photooxidation and shading in rice (*Oryza sativa* L.)

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Abstract

Forty-four genotypes from the rice germplasm were identified under photoinhibition/photooxidation and shade conditions and divided into four basic types: (*1*) cultivars tolerant to both photooxidation and shading, (*2*) cultivars tolerant to photooxidation but sensitive to shading, (*3*) cultivars tolerant to shading but sensitive to photooxidation, and (*4*) cultivars sensitive to both photooxidation and shading. Photosynthetic characteristics of a cultivar tolerant (cv. Wuyugeng 3) and a cultivar sensitive (cv. Xiangxian) to photooxidation and shading were compared. The photochemical efficiency (F_v/F_m) of photosystem 2 (PS2) and the content of PS2-D1 protein in the tolerant cultivar Wuyugeng 3 decreased less under photooxidative conditions as compared with Xiangxian. Under similar conditions, superoxide dismutase was induced rapidly to a higher activity and the active oxygen (O^-) built up to a lower level in Wuyugeng 3 than in Xiangxian. Net photosynthetic rate (P_N) decreased by 23 % in Wuyugeng 3 *vs.* 64 % in Xiangxian. Shading (80 %) during the booting stage caused only small decreases (7-13 %) in ribulose-1,5-bisphosphate carboxylase activity and P_N in Wuyugeng 3 but severe decreases (57-64 %) were observed in Xiangxian which corresponded to the decreases in grain yield of the two cultivars (38 and 73 %, respectively). We describe a simple and effective screening method and physiological basis for breeding crops for enhanced tolerance to both high and low irradiance.

Additional key words: chlorophyll; grain; irradiance; net photosynthetic rate; photooxidation; photosynthetic characteristics; photosystems 1 and 2; ribulose-1,5-bisphosphate carboxylase; shoot dry mass; superoxide dismutase.

Introduction

In the field, crops endure a wide range of fluctuating radiation. These variations result in excessive high or low irradiance, which directly limits photosynthetic potential and results in decreased yield. In a cereal crop such as rice, the irradiation during the later developmental stages is vital for grain filling and final yield. Early leaf senescence in leaves caused by photooxidation due to a combination of high irradiance and low temperature severely restrict yield potential. Inability to use radiation effectively for photoassimilation during cloudy day also reduces yield. Therefore, studying the physiological basis of photosynthetic tolerance to excessive high irradiance and shading is an important strategy for obtaining stable, high yielding rice cultivars. An ideal cultivar should be well adapted to both high and low irradiance, which would ensure a stable yield over a wide range of radiation conditions during the growing season. Combining a good plant-type and heterosis with the ability to adapt to a wide range of irradiances might be an effective approach for screening for high-yielding rice in a breeding program.

During the long history of evolution, rice crops in the different geological regions have evolved unique photosynthetic characteristics in response to specific radiation environments (Wang 1986). Thus, rice exhibits varying degrees of photosynthetic adaptation to strong or low irradiance, or to a wide range of irradiance (Jiao *et al.* 1996). Earlier, we developed a simple and effective technique for screening rice germplasm for tolerance to photooxidation and shading (Jiao 1992, Jiao *et al.* 1996). In this study, this simple method was used to screen 44 rice cultivars from germplasm collections for their tolerance to high and low irradiance. The major objectives of this study are to use a simple and effective technique in breeding program to screen rice cultivars adapted to a wide range of irradiance and to elucidate the physiological and biochemical bases of this adaptation.

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Abbreviations: Chl – chlorophyll; F_m – maximum Chl fluorescence; F_v – variable Chl fluorescence; F_v/F_m – PS2 photochemical efficiency; P_N – net photosynthetic rate; PR – photorespiration rate; PS – photosystem; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase; SOD – superoxide dismutase.

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Materials and methods

Plants and growth conditions: In recent years, forty-four rice cultivars from germplasm collections were identified repeatedly and grown from seeds in 10 000 cm^3 pots. Five individual plants per pot were grown in a net out-door room in Nanjing. Plants were watered and fertilised regularly during the growing season (May 1 to October 15). Average temperature varied from 21 to 27 °C and the diurnal temperature difference was from 7.1 to 8.7 °C. Chemical fertiliser was applied at the tillering and boot stages as a combination of 2.0 g N, P_2O_5, and K_2O per pot as basal dressing, and 0.5 g N and K_2O per pot as top dressing. The maximum photosynthetically active radiation (PAR) at noon on a sunny day in July-August was approximately 1 800 µmol m^{-2} s^{-1}.

Screening for tolerance to shading: For this screening, low irradiance was imposed for 14 d at the elongation-boot stage (*i.e.*, during the rapid growing stage with the production of the last two leaves on the stem). Uniform plants of each cultivar were divided into two groups (10 pots per group). One group of plants was maintained under natural radiation and the other placed under a shade area with 80 % reduction in irradiance (Jiao *et al.* 1996). The leaves and stems were cut at the ground, dried (80 °C, 72 h), and weighed after 14 d of shade. The total shoot dry mass of the plants that were grown under natural radiation (control plants) was taken as 100 %, and the percentage of the dry mass of the shade plants relative to the control plants was used as an index of shading tolerance. Based on the index, cultivars with a relative percentage above 50 % after shade were classified as tolerant to shading.

Grain yield was measured by harvesting all plants in 10 pots, weighing the grain, and calculating grain mass per plant or per pot. Other traits were obtained from 10 hills selected randomly from 10 pots. Plant height was measured from the soil surface to the tip of the head. Grains/panicle and filled grains/panicle were determined by random counting. Seed set was expressed as the ratio between the filled grains and the grain per panicle. Seed size was determined by weighing 1 000 filled grains randomly selected. Panicle length was measured from the bottom to the tip of panicle. Analysis of variance was performed and T-test was used to identify significant differences in all traits.

Screening for tolerance to photooxidation: Photooxidation treatments were also carried out at the booting stage (Jiao 1992). Five detached mature leaves from rice plants grown under natural irradiance were cut and immersed in water containing equilibrated ambient levels of CO_2 and O_2 in a white tray (23×17 cm). After 2 h, the O_2 concentration increased to about 265 µM and CO_2 concentration decreased to minimum due to photosynthesis of the leaves. Leaf blades kept flat by an overlaid transparent glass plate to prevent them from floating were exposed to natural sunlight. Water temperature was equilibrated with air temperature during the day (maximum temperature 35 °C) and night (25 °C). Tolerance to photooxidation was determined by the decline in chlorophyll (Chl) content and by the visible score of leaf colour after six days.

Chl content in leaves was measured according to the method of Wellburn and Lichtenthaler (1984), after extraction in 16.48 M ethanol.

Net photosynthetic rate (P_N) of intact leaves was monitored with a *LI-6200* portable photosynthetic measurement system (*Li-Cor*, Lincoln, NE, USA) at 30 °C, 350 µmol(CO_2) mol^{-1} under varying irradiance. To monitor the effect of photooxidation conditions on the overall photosynthetic capacity, O_2 evolution was measured in detached leaves with an *SP-2* oxygen electrode (*Sinica Academy*, Shanghai, China) at 1 000 µmol m^{-2} s^{-1} PAR and 28 °C.

Superoxide dismutase (SOD) activity was assayed by the method of Giannopolitis and Ries (1977). Leaf tissue was thoroughly ground with a mortar and pestle in an ice bath, until no fibrous residue could be seen. The grinding medium [4 000-6 000 cm^3 kg^{-1}(f.m.)] consisted of 0.1 M K_2HPO_4 and 0.1 mM EDTA (pH 7.8), plus homogenising glass beads. The homogenate was centrifuged twice at 13 000×*g* for 10 min in a *Sorvall RC2-B* refrigerated centrifuge at 0-5 °C. The supernatant is referred to as the crude SOD extract. SOD activity was assayed by its ability to inhibit the reduction of nitro blue tetrazolium (NBT) by the O_2^- generation system xanthine oxidase–xanthine. The reaction (final volume of 3.2 cm^3) was started by the addition of sufficient xanthine oxidase to produce 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 the initial rate of NBT reduction.

Determination of O_2^-: The content of O_2^- was measured according to the method of Wang and Luo (1990). Leaf segments (about 5 g fresh mass) were immediately homogenised 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 5 000×*g* for 10 min at 0 to 4 °C, 0.9 cm^3 phosphate buffer, and 0.1 cm^3 10 mM hydroxylamine hydrochloride was added in 1 cm^3 of supernatant. This mixture was incubated at 25 °C for 20 min. 0.5 cm^3 of incubated mixture was injected into 0.5 cm^3 17 mM *p*-aminobenzoic acid and 0.5 cm^3 17 mM α-napthaleneamine, at 25 °C for 20 min. The developing solution was shaken with equal volume of *n*-butanol and subsequently separated into two phases. This phase with *n*-butanol phase was taken out and measured at 530 nm. The phosphate buffer without the sample was used as control. If there were large amounts of chlorophyll in the sample, ethyl ether replaced *n*-butanol and the mixture was centrifuged at 1 500×*g* for 5 min. The absorbance of water phase at 530 nm was then measured. O_2^- production was checked up from the standard curve of developing NO_2^- reaction. O_2^- production rate = O_2^- production/reaction time×the amount of protein [mmol(O_2^-) kg^{-1}(protein) s^{-1}].

PS2 activity of isolated thylakoids was measured with a polarographic oxygen electrode as described by Shyam and Sane (1990). The reaction mixture contained 50 mM Tricine-KOH (pH 7.0), 5 mM $MgCl_2$, 1 mM $K_3Fe(CN)_6$, 10 µM 2.5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB), and chloroplast suspension at a final concentration of 5 g(Chl) m^{-3}.

PS1 activity: Isolation of intact chloroplasts and measurements of PS1 with an oxygen electrode were described previously. The reaction mixture contained Tris-HCl 50 mM (pH 7.8), NaCl 10 mM, NH_4Cl 50 mM, 2,6-dichlorophenolindophenol (DCIP) 20 µM, methyl-viologen (MV) 50 µM, 3-(3,4-dichlorophenyl)-1,1-dimethyl-urea (DCMU) 10 µM, and chloroplast suspension equivalent to 50 mg of Chl.

RuBPCO activity: RuBPCO was prepared and its activity was determined according to Wei *et al.* (1994) and Kung *et al.* (1980), respectively. Fresh leaf segments (0.5 g fresh mass) were cut into small pieces and immediately homogenised in a pre-cooled pestle and mortar with acid-washed quartz sand and 2.5 cm^3 extraction medium containing 50 mM Tris-HCl, pH 7.5, 1 mM $MgCl_2$, 5 mM dithiothreitol (DDT), and 20 g insoluble polyvinylpyrrolidone (PVP). After mixing the filtrate thoroughly, a 40-mm^3 aliquot was transferred to a small test tube to estimate soluble protein. The homogenate was clarified by centrifugation at 10 000×*g* for 10 min. The clear supernatant was decanted slowly and used for enzyme activity. All these steps were carried out at 4 °C.

D1 protein determination: ^{14}C-atrazine [6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5- triazine-2,4-diamine] binding analysis was conducted according to Wu and Shen (1990) and Wu (1991). A stock solution of ^{14}C-atrazine [9.25×10^{11} Bq mol^{-1}] was diluted with buffer to 30, 60, 90, 150, 210, and 300 µmol m^{-3}. A 10-mm^3 aliquot of each of the above solutions was mixed with 1 cm^3 of chloroplast suspension. The mixture was heat-preserved for 5 min and centrifuged for 5 min at 16 000×*g*. Supernatant (0.5 cm^3) was added to 4.5 cm^3 of scintillation solution, 12 mM 2,5-diphenylozazol (PPO) + 0.1 mM 1,4-di-[2'-(5'-phenyloxazolyl)]-benzene (POPOP) methylbenzene : *Triton X-100* (2 : 1, v/v). The final mixture solution was determined by an aliquot scintillation counter. The content of D1 protein was expressed as the reciprocal of Chl to bound ^{14}C-atrazine. The affinity of thylakoids to ^{14}C-atrazine (Kb) was calculated as described by Kyle *et al.* (1984).

PS2 photochemical efficiency, F_v/F_m was measured by using a portable Chl fluorometer (*FMS-2, Hansatech Instruments*, UK) as described by Guo *et al.* (1994). The leaves were dark-adapted for 5 min before the measurement of the initial fluorescence yield (F_0) in weak modulated radiation (0.12 µmol m^{-2} s^{-1}, 600 Hz) and the maximum fluorescence yield (F_m) emitted during a saturating radiation pulse (4 000 µmol m^{-2} s^{-1}, 0.8 s). Variable Chl fluorescence (F_v) was calculated as $F_v = F_m - F_0$.

Results

Identification of rice cultivars tolerant to photooxidation and shading: Forty-four rice cultivars were screened for sensitivity or tolerance to photooxidation and shading (Fig. 1). The reduction in Chl content after 6 d of photooxidative conditions (high irradiance, low CO_2) was used as an index of tolerance to photooxidation while the reduction in shoot dry matter accumulation after 14 d of shading (20 % of normal irradiance) at the booting stage was used as an index of shading tolerance. A cultivar with a shading-induced reduction of less than 50 % in dry matter accumulation was considered tolerant while a cultivar with reduction of more than 50 % was considered as sensitive. A visible rating of leaf greenness after subjecting to photooxidative conditions was also used to score tolerance to photooxidation. Six cultivars (Wuyugeng 3, Yayou 2, Zhuzhiping, R3043, YueA×34077, 02428) received a rating of 1 (whole leaf remaining green) or 2 (with yellowish tip), and were identified as photooxidation-tolerant cultivars. All these cultivars showed a less than 30 % reduction in Chl content after treatment (Fig. 1). Compared with the plants grown under natural irradiation, 20 cultivars exhibited reduction of less than 50 % in shoot dry mass after shading and these cultivars were identified as shade tolerant.

A plot of the shade tolerance index *versus* the index of photooxidation tolerance showed that these 44 rice cultivars may be divided into four groups: (*1*) cultivars tolerant to both photooxidation and shading, (*2*) cultivars tolerant to shading but susceptible to photooxidation, (*3*) cultivars tolerant to photooxidation but susceptible to shading, and (*4*) cultivars susceptible to both photooxidation and shading (Fig. 1). The *japonica* cv. Wuyugeng 3, a high yielding cultivar widely cultivated in Jiangsu province, is tolerant to both photooxidation and shading, whereas the *indica* cv. Xiangxian, which has been recently phased out in Jiangsu province, is highly susceptible to both photooxidation and shading. Thus, Wuyugeng 3 and Xiangxian were chosen for further study to check the physiological and biochemical bases for their different response. The majority of tested cultivars (50 %) were susceptible to both photooxidation and shading while only a few cultivars were tolerant.

Photosynthetic characteristics and tolerance to photooxidation: Under photooxidation conditions, the degree of photooxidation damage is often assessed by the decrease in Chl content. During exposure to photooxiditive conditions, the tolerant cv. Wuyugeng 3 exhibited a very small decrease in Chl content (12 %) while the Chl content of the sensitive cv. Xiangxian decreased drastically after 3 d of treatment (Fig. 2). By day 6, Chl content in Xiangxian was almost totally degraded. These results are consistent with those obtained in the initial screening (Fig. 1).

Photochemical activities, P_N, production of oxygen radical, and activity of the oxygen radical scavenging enzyme SOD were also measured simultaneously during the photooxidation treatment. PS1 activity in the tolerant cv. Wuyugeng 3 increased during the first day of treatment and decreased progressively thereafter (Fig. 2). On the other hand, the PS1 activity in Xiangxian decreased immediately. Also, the degree of decrease was always greater in Xiangxian than in Wuyugeng 3. In contrast, PS2 activity decreased immediately after the treatment and the decrease was accelerated after the third day of treatment (Fig. 2). Throughout the entire treatment period, the tolerant cv. Wuyugeng 3 was able to maintain higher (20 %) activities than the sensitive cv. Xiangxian. Comparison of the rates of change between PS1 and PS2 showed that PS2 is more prone to photooxidative damage. The increase in PS1 activity in Wuyugeng 3 in the first day of treatment may be related to the com-

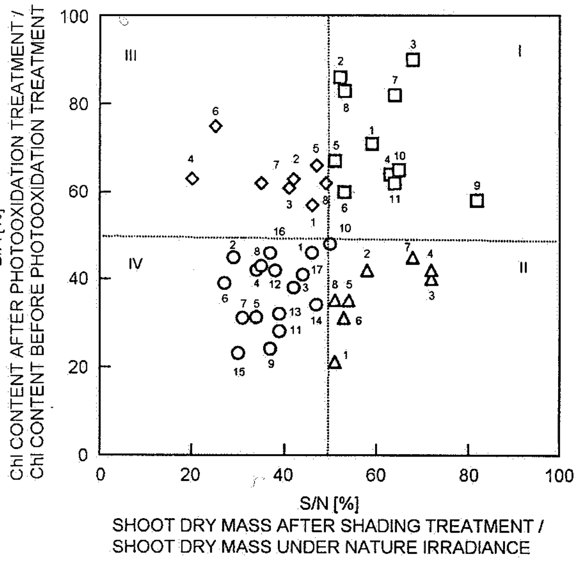

Fig. 1. Effect of shading and photooxidation treatment on shoot dry mass accumulation and chlorophyll (Chl) content in 44 rice cultivars: I – tolerant to both photooxidation and shading, II – tolerant to shading and susceptible to photooxidation, III – tolerant to photooxidation and susceptible to shading, IV – susceptible to both photooxidation and shading. J: *japonica*, I: *indica*. In brackets the former value is shown as % of S/N, the latter one as % of B/A.

□ 1 Wuyugeng 3 (J) (59, 71)
2 Yayou 2 (J) (52, 86)
3 02428 (J) (68. 90)
4 Yue A×R3044 (I) (63, 64)
5 Liuqianxins×JW207 (J) (53, 60)
6 liuqianxins (J) (51, 67
7 Yue A×34077 (I) (64, 82)
8 R3034 (I) (64, 82)
9 Yue A×R3043 (I) (82, 58)
10 Yue A×J204 (J) (65, 65)
11 JW201 (J) (64, 62)

Δ 1 Jinggang (I) (51, 21)
2 Shanyou 63 (I) (58, 42)
3 YueA×R3035 (I) (53, 31)
4 Yumidao (I) (72, 42)
5 320500 (I) (54, 35)
6 Yue A×R3034 (I) (72, 40)
7 Yue A×R3049 (I) (68, 45)
8 YueA×30152 (J) (51, 35)

◊ 1 Peiai64s×9311 (I) (46, 57)
2 R3034 (I) (42, 63)
3 34077 (I) (41, 61)
4 NJ 14 (I) (20, 63)
5 Liuqianxins×JW201 (J) (45, 67)
6 Zhuzhiqing (J) (25, 75)
7 Minghui 63 (I) (35, 63)
8 9311 (I) (49, 62)

○ 1 276 (J) (46, 46)
2 JW 204 (J) (29, 45)
3 Peiai 64s×Zhongjing 32 (I) (42, 28)
4 R3049 (I) (34, 42)
5 R3035 (I) (34, 30)
6 Xiangxian (I) (27, 49)
7 YueA×R437 (I) (31, 31)
8 YueA×32050 (J) (35, 43)
9 Zhenxian 97 (I) (37, 24)
10 JW207 (I) (50, 48)
11 Peiai64s×JW201 (J) (39, 29)
12 R437 (J) (38, 42)
13 R3044 (I) (39, 32)
14 YueA (I) (47, 37)
15 Zhongjing (J) (30, 23)
16 Peiai 64s (I) (37, 46)
17 YueA×42525 (I) (44,41)

pensatory effect of PS2. Consistent with the changes in Chl content and photochemical activities, the photosynthetic rate of Xiangxian, as measured by O_2 evolution in CO_2-saturated conditions, decreased rapidly with time and by day 6 only 5 % of the initial rate remained (Fig. 2). In contrast, P_N of Wuyugeng 3 exhibited a mild decrease upon treatment and after 6 d there remained still more than 55 % of the photosynthetic capacity.

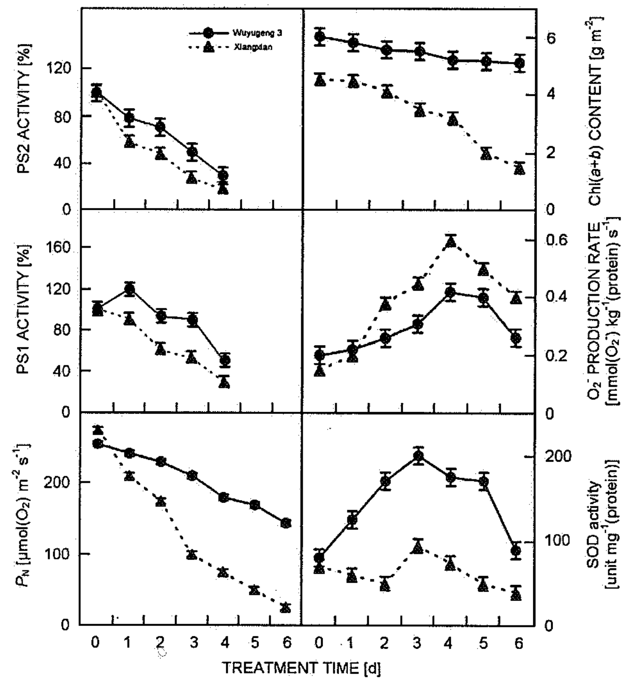

Fig. 2. Changes in photosystem (PS) 1 and 2 activities, net photosynthetic rate (P_N), chlorophyll (Chl) content, O_2^- production rate, and SOD activity in Wuyugeng 3 and Xiangxian under photooxidative conditions.

One of the immediate products of photoinhibition/photooxidation is formation of oxygen radicals, due to the inability of the photosynthetic machinery to use the excess photons. However, plants have evolved various mechanisms to defend against such stress and one such mechanism is an oxygen radical scavenging system (Salin 1988). A measure of scavenging activity and the amount of oxygen radical produced under photooxidative conditions is useful for determining the plant's defensive capability to such stress. In Xiangxian, the amount of O_2^- increased rapidly from 0.2 to 0.6 mmol(O_2^-) kg^{-1}(protein) s^{-1} in the first four days and decreased thereafter (Fig. 2). In contrast, Wuyugeng 3 showed a mild increase, from 0.15 to 0.40 mmol(O_2^-) kg^{-1}(protein) s^{-1} before it decreased. The activity of SOD, the key enzyme in the scavenging of O_2^-, increased rapidly in Wuyugeng 3 during the first three days, from 90 to 215 unit mg^{-1}(protein), and decreased thereafter (Fig. 2). On the other hand, there was no immediate increase of the enzyme activity in Xiangxian in the first two days, and the increase on the third day was small. These results suggest that, as compared with Xiangxian, the tolerant cv. Wuyugeng 3 possesses a much stronger defence mechanism against oxygen radicals in response to the stress.

To further elucidate the physiological basis of stability of PS2 activity in the photooxidation tolerant cultivar, PS2 activity, PS2 photochemical efficiency (F_v/F_m), and D_1 protein content in the two cultivars were measured after treating with CO_2-free air containing 1 kPa O_2 at high irradiance for 4 h. P_N, photorespiration rate, and RuBPCO activity were measured simultaneously. Changes in F_v/F_m, PS2 activity, and P_N in the two cultivars followed the same trend, but the indexes dropped less in Wuyugeng 3 than in Xiangxian (Table 1) after the photoinhibition treatment. No significant change of RuBPCO activity was observed. These results show that photoinhibition in rice is associated with changes in electronic transport activity and photoenergy conversion, but it is not associated with the activity of RuBPCO, the key enzyme of Calvin cycle. Maintaining capacity of D1 protein in PS2 reaction centre may be an important

Table 1. Changes in photosynthetic characteristics of attached leaves in Wuyugeng 3 and Xingxian after 3 h of exposure under photooxidative conditions. Measurements were made on the top two leaves. Means±SE ($n = 6$). NC = no change. Photooxidative conditions: Leaf chamber was flushed with CO_2 free air containing 1 kPa O_2 at 1 000 µmol m^{-2} s^{-1} for 3 h. The correlation coefficients between net photosynthetic rate and PS2 photochemical efficiency, D1 protein content, and PS2 activity were 0.88**, 0.90**, and 0.76*, respectively. $^{*, **}$ significant at 0.05 or 0.01 probability levels, $n = 6$.

	Wuyugeng 3 Control, C	Treatment	% of C	Xiangxian Control, C	Treatment	% of C
PS2 photochemical efficiency (F_v/F_m)	0.84±0.04	0.63±0.02	75	0.84±0.04	0.43±0.02	52
PS2 activity [nmol(O_2) kg^{-1}(Chl) s^{-1}]	246.10±4.30	197.40±6.40	80	236.30±6.10	146.70±4.40	62
Dl protein [mmol(^{14}C-atrazine) kg^{-1}(Chl)]	1.93±0.12	1.54±0.06	80	1.89±0.09	1.08±0.05	57
Net photosynthetic rate [µmol(CO_2) m^{-2} s^{-1}]	24.92±1.62	20.26±1.26	81	24.42±1.88	12.32±0.94	51
Photorespiration rate [µmol(CO_2) m^{-2} s^{-1}]	8.56±1.08	8.32±0.68	NC	8.22±0.82	7.98±0.82	NC
RuBPC activity [mmol(CO_2) kg^{-1}(Chl) s^{-1}]	147.83±6.81	135.94±3.42	92	144.83±6.47	134.50±6.08	93
RuBPO activity [mmol(O_2) kg^{-1}(Chl) s^{-1}]	38.44±3.47	38.44±3.47	NC	31.36±2.81	31.36±2.81	NC

physiological and biochemical index of a cultivar tolerant to photooxidation.

Photosynthetic characteristics and tolerance to shading: Under shade, the dry mass of Wuyugeng 3 decreased by only 41 % by day 14, while that of Xiangxian decreased by 73 % (Fig. 1). So Wuyugeng 3 is also a shade tolerant cultivar and Xiangxian is a sensitive one. The yield (g per pot) of Wuyugeng 3 was 73 % of the control and that of Xiangxian was 32 % after shading for 14 d (Table 2). The productivity was reduced more with the increase in days of shading. The reduction of yield was mainly related to a decline in grain size (Table 2).

The parameters of irradiance-P_N curve in Fig. 3 indicate that the quantum efficiency in Wuyugeng 3 was 0.06, which was higher than in Xiangxian (0.04). The maximum P_N in Wuyugeng 3 under shade decreased only by 10 % as compared with that in natural irradiance, however, the maximum P_N of Xiangxian decreased by 57 %. These results suggest that the shade-tolerant cultivar has a high photosynthetic efficiency and maximum P_N under shade, which is consistent with the change in photosynthetic productivity and yield. To elucidate the physiological basis of cultivar difference in photosynthetic performance, PS2 activity and photosynthetic enzyme activity in Wuyugeng 3 and Xiangxian were determined under natural irradiance and shade (Table 3). The PS2 activity and RuBPCO activity of the two rice

Table 2. Effects of shading on grain yield and yield components of Wuyugeng 3 and Xiangxian. Shading (80 % reduction in irradiance) was applied at booting stage for 21 d. Means±SE ($n = 3$). Correlation coefficient between grain yield per plant and seed size was 0.93. **, significant at $p = 0.01$, $n = 24$.

	Shade [d]	Plant height [cm]	Grains per panicle	Filled grains per panicle	Seed set [%]	Seed size [mg seed^{-1}]	Panicle length [cm]	Panicles per plant	Grain yield [g plant^{-1}]	[g pot^{-1}]
Xangxian	0	93±4	144±18	103±16	72	20.5±1.3	23.8±2.6	7.3±1.8	13.1±1.8	56.8±5.8
	3	96±7	120±34	91±30	76	17.0±2.1	22.5±2.8	4.9±2.0	8.8±1.3	33.4±2.1
	14	85±5	111±30	67±22	60	10.5±1.4	23.2±2.5	4.6±0.5	5.0±0.8	18.2±3.1
	21	80±2	101±28	58±24	57	9.8±2.3	22.5±2.9	3.5±1.0	3.6±0.5	15.1±1.3
Wuyugeng 3	0	84±4	102±11	97±11	95	24.7±4.1	14.4±2.4	4.7±1.1	15.0±2.3	68.2±3.7
	3	87±2	101±27	94±27	94	26.9±3.2	15.2±2.1	4.1±1.1	12.9±1.8	51.4±5.4
	14	84±3	72±20	52±18	73	18.8±2.1	13.7±1.7	3.7±0.6	8.7±0.6	49.5±2.8
	21	82±6	64±16	46±15	71	18.0±3.2	13.4±1.2	3.0±1.0	8.3±0.9	42.4±2.9

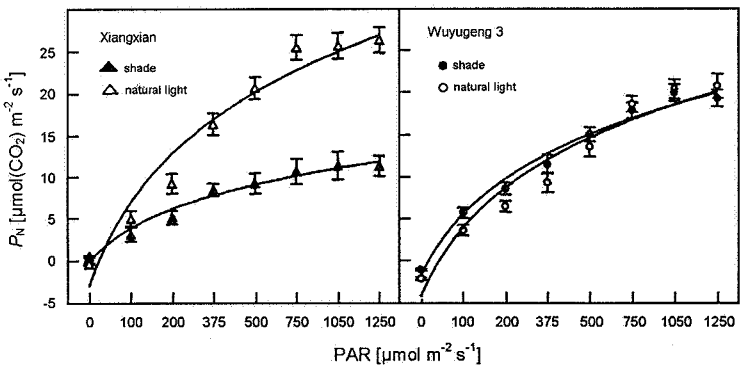

Fig. 3. Dependence of net photosynthetic rate (P_N) on irradiance (PAR) in attached leaves of Wuyugeng 3 and Xiangxian under natural irradiance or shade conditions.

Table 3. Chages in net photosynthetic rate (P_N) [μmol(CO$_2$) m^{-2} s^{-1}] and activities of photosystem 2 (PS2) [nmol(O$_2$) kg^{-1}(Chl) s^{-1}] and ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) [mmol(CO$_2$) kg^{-1}(Chl) s^{-1}] of Wuyugeng 3 and Xiangxian in natural irradiance (N) or after shade (S; 80 % reduction in irradiance) was applied at the booting stage for 14 d. Measurements were made on the top two leaves. Means±SE ($n = 3$). Correlation coefficient between P_N and RuBPCO activity was 0.87**. *, ** significant at 0.05 or 0.01 probability levels, respectively. $n = 12$.

Cultivar	P_N			PS2			RuBPCO		
	N	S	S/N	N	S	S/N	N	S	S/N
Wuyugeng 3	20.2±2.3	18.1±2.1	90	247.3±4.3	215.3±4.0	87	532.3±24.5	457.0±22.9	86
Xiangxian	26.0±3.4	11.2±1.9	43	236.2±3.5	181.8±2.7	77	521.4±23.3	192.9±8.5	37

cultivars decreased during 14 d of shade, but the decline in P_N was more consistent with that of RuBPCO activity. These results suggest that photosynthetic performance of shade-tolerant cultivars is related to the stability of RuBPCO activity. This mechanism needs further study.

Discussion

Plants adapted to sun and shade belong to two ecotypes, which have different photosynthetic characteristics (Boardman 1977). Photosynthetic adaptation and acclimation of plants to their environment has been studied extensively at the levels of plant canopy, whole plant, leaf, and organelle (Lichtenthaler *et al.* 1983, Givnish 1988, Murty and Deys 1992). The mechanisms of photosynthetic adaptation to high and low irradiance lay mainly in changes in the components of photosystems (Chow *et al.* 1991) and in activities of enzymes related to photosynthetic carbon metabolism (Seemann 1989). In last decade, studies of related mechanisms were mostly focused on the photochemical processes. Under excessive high irradiance, the primary site of photoinhibition may be located in the PS2 reaction centres, and related to the rapid turnover of the D1 protein under radiation stress, that leads to its depletion and loss of PS2 activity (Aro *et al.* 1993, Long *et al.* 1994). During evolution, many endogenous adaptive reaction systems have been developed that protect the plants against photoinhibition. Among them are the xanthophyll cycle for thermal dissipation of excess absorbed photons and the protective systems of scavenging reactive oxygen species including the antioxidant molecules and the scavenging enzymes (Demmig-Adams and Adams 1992, Foyer *et al.* 1994, Niyogi 1999, Xu *et al.* 2000). Under low irradiance, photosynthetic adaptation is expressed as a rapid increase in the light-harvesting complex (LaRoche *et al.* 1990). The topic has been studied intensively at the molecular level, mainly in lower plants. Only little attention has been paid to the agricultural crops. The above discoveries provide useful basis for understanding the mechanism of photosynthetic adaptation to adverse irradiance in rice crop.

Rice crops grow in an open ecological system in the field. Due to the long-term acclimation to growth irradiance in the nature, remarkable cultivar differences in photosynthetic adaptation in rice were found (Tu *et al.* 1988, Huang *et al.* 1989). For mass screening of rice germplasms tolerant to photoinhibition/photooxidation, we have developed a simple and effective identification technique. Thus we found that a *japonica* cv. was usually more tolerant than an *indica* one (Jiao 1992). This technique was applied in some laboratories, even for screening other crops (Gu *et al.* 1999, Wang *et al.* 2000, Xin *et al.* 2000). Then, we used the shade technique for mass screening of rice germplasms tolerant to shading (Jiao *et al.* 1996). In recent years, we combined both above techniques for screening cultivars tolerant to photooxidation and shading. Forty-four genotypes from rice germplasms were identified and divided into four groups, distinguishing especially cultivar types tolerant to both photooxidation and shading (Fig. 1). The comparison of different cultivar types showed that the short-time photoinhibition (3 h) affected more the photochemical reactions than the carbon metabolism. The tolerant cultivar showed higher maintaining capacity of D1 protein and PS2 photochemical efficiency, thus exhi-biting less photoinhibition of photosynthesis (Table 1). Previous studies demonstrated that D1 protein encoded by plastid gene was more important for the mechanisms of photoinhibition than alternative protective strategies, namely, xanthophyll cycle, ΔpH, or SOD (Anderson *et al.* 1995, Ji and Jiao 2000, Jiao and Ji 2001). Under long-time photooxidative conditions (days), SOD activity was larger and of longer duration and the $O_2^{-\cdot}$ generation and accumulation was lower in the tolerant cultivar. Therefore it exhibited a slower reduction in Chl content and leaf colour, which might be the reason why the rice cultivars tolerant to photooxidation did not age earlier when they endured stress at the late developmental stages. In addition, after shading for 14 d the long-time low irradiation affected carbon metabolism more than photochemical reactions, and the activity of RuBPCO in shade-tolerant cultivars was high (Table 3). This demonstrated that the rice cultivars tolerant to a wide range of irradiance maintained a high activity of PS2 and RuBPCO, which might be the physiological basis for adaptation to adverse irradiance. These results were only a preliminary clue for further research. Recently, progress in research on photoinhibitory characteristics of rice has been made (Black *et al.* 1995, Murchie *et al.* 1999, Jiao and Ji 2001), but no much information was obtained as concerns cultivar differences in tolerance to shading. The expression of *CAB* gene encoding PS2- and PS1-associated Chl *a/b* binding protein that adjusts absorption of photons was studied by Wehmeyer *et al.* (1990). In carbon metabolism, RuBPCO activase and RuBPCO binding protein may enhance the activation and protection of RuBPCO (Ernstsen *et al.* 1999).

In order to enhance photosynthetic productivity of rice, the breeders have successfully assembled the different adaptation to irradiance derived from both parents into hybrid rice by genetic manipulation. A high-yielding rice cultivar Le/qi adapted to a wide range of irradiation in China has been developed by crossing the *japonica* cv. Lemont tolerant to high irradiance with the *indica* cv. Qiguizhao tolerant to shade (Tu *et al.* 1993). Our present findings also show that many hybrid rice types were tolerant to a wide range of irradiance (Fig. 1).

To sum up, the mass screening for rice cultivars tolerant to photooxidation and shading can be done by this simple technique, which is recommendable for conventional breeding. We hope that this technique will be improved in future by the breeding practice.

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