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Full Length Research Paper

Variation of photosynthetic tolerance of rice cultivars (*Oryza sativa* L.) to chilling temperature in the light

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Forty-two genotypes from the rice germplasm (*Oryza sativa* L.) were identified under chilling temperature in the light at bud, seedling and booting stages and divided into three basic types; cultivars tolerant to chilling in the light such as *japonica*, cultivars sensitive to chilling in the light such as *indica* and cultivars that have intermediate tolerance to chilling in the light such as hybrid rice cultivar. Photosynthetic characteristics of two cultivars tolerant (c.v. Taipei309 and Wuyujing3), two cultivars sensitive (c.v.CA212 and Pusa) and two intermediated tolerant (c.v. Liangyoupeijiu and Shanyou63) to the chilling treatment in the light were compared. The results showed that, compared to the rice varieties chilling tolerant rice, the sensitive ones *indica* exhibited a significant inhibition of maximum photosynthetic rate (Pm) and a decrease in the photochemical efficiency of photo-system 2 (PS2)(Fv/Fm), which led to the accumulation of AOS and decrease of Chl content. Interestingly, the ratios of ASA/DHA and GSH/GSSG showed similar changes as those with the performance of chilling tolerance, which indicated that ASA/DHA cycle might be an important protecting strategy in chilling tolerance, especially for the middle tolerant ones. We describe a simple and effective screening method and physiological basis for breeding crops for enhanced tolerance to chilling temperature in the light.

Key words: Rice, chilling tolerance, photosynthetic rate, photo-system, chilling stress, photooxidation, gluthione, ascorbate.

INTRODUCTION

Indicia hybrid rice is grown on more than 1 trillion hectares (ha), making about 55% of the total rice growing area in China. Its yield is about 20% higher than that of conventional rice cultivars in China (9 - 10 t /ha or 0.9-1.0 Kg/m^2 (Cheng and Min, 2000; Cheng and Zhai, 2000). However, *indicia* hybrid and *indica-japonica* hybrid rice are more likely to suffer from low temperature during the whole developmental stages according to recorded observations over many years. Especially, the rice met with low temperature during the late developmental stages often result in early aging, which seriously restricts the potential for heterotic vigor.

Rice crops grow in an open ecological system in the field. Studies on chilling tolerance in rice begun in Japan, followed by reports in China, mostly from Yungui Highland of China (Li et al., 2006). Because these researches used empty seed or poor seed-setting rate as indexes for chilling identifications, it would take the whole growth stage to complete the identification. Furthermore, seed-empty rate of rice was easily influenced by the rice inheritance and the environment. As rice covers many ecological regions in the world, the chilling identification of different ecotypes from different ecological regions is often inconsistent even for one rice variety.

It found that the performance of photo-inhibition, photo-oxidation and early aging in rice at low temperature and high light intensity in late development stages were closely related (Jiao et al., 2003). On sunny days at a temperature above 25°C, the reaction center of photosystem 2 (PS2) exhibited a dynamic change on reversible inactivation and down-regulation in order to reduce photo-inhibition damages under intense irradiance at the noon

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with low temperature. The photo-damage and early aging caused by PS2 were related to the degradation of PS2-D1 protein and the inhibition of endogenous protection systems such as the xanthophylls cycle and enzymes scavenging active oxygen (Jiao and Ji, 2001). To complement the dissipation mechanisms mentioned above and counteract the oxidative pressure imposed by ROS formation, plants possess a multi-level antioxidant system, consisting of small antioxidants like ascorbate, a-tocopherol and glutathione as well as a multitude of ROS scavenging enzymes (Asada, 2000). Unfortunately, the metabolism of the antioxidant of leaves on cultivars' difference in chilling-tolerance in rice has not yet been reported (Conklin, 2001).

In the study, 42 rice cultivars were used to identify the chilling tolerance and to study their effect of protection in photosynthesis under low temperatures in the light. Our study focused on the antioxidants for the role of chilling tolerance. It will provide the basis on the photosynthetic aspect of genetic approaches to rice chilling breeding.

MATERIALS AND METHODS

Plant

The rice seeds were from Jiangsu Academy of Agricultural Sciences. The 13 *japonica* rice cultivars included 9516, H45, Wuyujing 3, PEPC transgenic rice, Kitaake and Suhuxiangjing. The 7 *indica* rice cultivars were Yangdao6, Xiangxian, IR64 and Peiai64S. The 6 *japonica* hybrid rice cultivars include SZ601 and the 16 *indica* hybrid rice cultivars include Yueyou 938, Shanyou63, X07s/zihui100 and Liangyoupeijiu. These cultivars were selected as materials in Nanjing, China, during the years between 2003 and 2008. The rice seeds were sterilized in 5% H_2O_2 for 5 min, soaked in water for 24 h, incubated at 35℃ for 48 h and finally sowed by stage. Seedlings at similar developmental stages were transplanted into pots (5 hills per pot, 1 seedling per hill) and grown in an outdoor net-room. A completely randomized design with five replicates was utilized. Average temperature varied from 21℃ to 27℃, with daily temperature differences from 7.1℃ to 8.7℃. Chemical fertilizer was applied with a combination of 2.0 g N, 1.6 g P_2O_5 and 1.4 g K_2O per plot as basal dressing and 1.0 g N as top dressing at the tillering and booting stages. The soil type was paddy soil.

The treatment of chilling temperature on rice at different stages

According to the method developed by Li and Cheng, (2005), uniform sprouting seeds (the length of bud was 0.05 m and the root height was 0.1 m) at bud stage were placed on a white plastic tray (0.23 × 0.17 m) with filter paper soaked with water. Each tray was sown with 50 buds of one variety. All the cultivars were treated less than 4℃ for 5 d. The livability of treated buds was calculated. At seedling stage (6-week-old), uniform buds of rice cultivars were grown at growth champers at 28℃ L/26℃ D. The six-week old seedlings of each cultivar were then placed at 8℃ with a PPFD of 600 μmol/m^2 s for 2 d. The livability of these seedlings treated was again calculated. At booting stage, the rice plants were placed under 15℃ with a PPFD of 600 μmol/m^2 s for 5 d. After that, the treated plants were transported to outdoors in a screen house. Rice plants were watered and fertilized regularly. The seed-setting rate of the treated plants was measured after harvesting all the plants.

Photosynthetic rate (P)

Photosynthetic rate (P) of intact leaves in rice were monitored with a Li-Cor 6400 (Lincoln, Nebraska, USA) at 25℃ under varying irradiance according to the method of Li et al. (2002a).The gas source was compressed air (CO_2 concentration was 350 μmol mol^{-1}). The light source was halogen light source. Varying irradiances on leaf surface were obtained by regulating the distance between light source and leaf chamber. A layer of circulating water between leaves and the illumination source was maintained for heat insulation (keep at 25℃ and 60% relative humidity).The P under varying irradiation such as 0, 50, 100, 150, 200, 400, 600, 800, 1000 and 1200 μmol/m^2 s was measured respectively. The photosynthetic rate at each PPFD was surveyed with 4 to 6 repetitions. The photosynthetic light response curves were obtained by measuring the steady state rates under different PPFD. The photosynthetic CO_2 response curves were obtained by measuring the steady state rates under different CO_2 concentrations in ranges of 0 - 1000 μmol mol^{-1}.

Chlorophyll fluorescence parameters

Chlorophyll fluorescence parameters were measured using FMS-2 fluorescence meter (Hansatech, UK) and calculated according to Genty et al. (1989). The rice leaves were modulated measuring beam (0.12 μmol/m^2 s) to determine the initial fluorescence yield (Fo). Maximum fluorescence yield (Fm) was determined during a saturating photon pulse (4000 μmol/m^2 s). Variable Chlorophyll fluorescence (Fv) was calculated as Fv = Fm - Fo. Primary PS2 photochemical efficiency was expressed as Fv/Fm. Chlorophyll content in leaves was measured according to the method of Aron (1949).

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 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 then centrifuged and 5000 × g for 10 min at 0-4℃. Phosphate buffer (0.9 ml) and 10 mM hydroxylamine hydrochloride (0.1 ml) was added in 1 ml of supernatant. This mixture was incubated at 25℃ for 20 min. A half ml of incubated mixture was injected into 0.5 cm^3 17 mM p-aminobenzoic acid and 0.5 cm^3 17 mM α-naphthaleneamine at 25℃ 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 sample was used as control. If there were large quantity of chlorophyll in the sample, ethyl ether was used to replace n-butanol and the mixture was centrifuged at 1500 × g for 5 min. The absorbance of water phase at 530 nm was then measured. The production of O_2^- was compared to the standard curve of developing NO_2^- reaction.

Measurement of malonyldialdehyde (MDA)

Membrane lipid peroxidation was determined by the accumulation of membrane lipid peroxidation product-MDA according to the method of Heath and Packer (1968). The reaction between MDA (1 mol) and thiobarbituric acid (TBA, 2 mol) formed red-brown trimethine that can be detected quantitatively with spectrophoto-

meter. Leaf discs (0.5 g) were ground in a solution containing 5 ml of 10% trochloroacetic acid (TCA) and some quartz sand. The homogenate was centrifuged for 10 min at 3,000 × g to remove cell debris. Then 2 ml of supernatant was collected and further mixed with 2 ml of 0.67% TBA (w/v). After keeping in boiling water for 20 min and cooling fully, the mixture was centrifuged at 3000 × g again. Finally, the supernatant was measured at 532 nm and 600 nm with a spectrophotometer.

Measurement of H_2O_2

According to the method of Patterson et al. (1984), one gram of leaf blades was homogenized in 3 ml cold acetone. The homogenate was centrifuged for 10 min at 16000 × g. The supernatant (1 ml) was added with 0.1 ml of 20% $TiCl_2$ in concentrated HCl, 0.2 ml concentrated ammonia solution. The peroxidation product with Ti component was washed five times with acetone, drained and dissolved in 3 ml 1 M H_2SO_4. The absorbance of the solution was measured at 410 nm and quantified according to the standardization curve of H_2O_2 produced by a similar procedure.

Fatty acids

Fatty acids were analyzed according to method of Yu and Su (1996). Lipids were methy-esterified in solution with 0.4 M KOH and benzene-petroleum ether (1:1, v/v). The fatty acid methyl esters were separated by gas chromatography (Shimadzu GC-17A, Japan) equipped with a hydrogen flame detector and a capillary column SP-2330 (15 m in length and 0.32 cm in i.d.). The column was iso-thermally run at 165°C and the detector was held at 250°C. The standard reagents of fatty acids were purchased from Sigma (US).

Total glutathione and glutathione disulphide content

Glutathione was determined by enzymatic assay using 0.1 g ground frozen leaf material in 1 ml extraction buffer containing 6% $HClO_4$ and 0.2mM DTPA (Luwe et al., 1993). After centrifugation at 14,000 × *g* for 10 min, the supernatant was used to measure the content of glutathione (as GSH) and GSSG by determining the rate of absorbance increase at 412 nm for 120 sec (n = 7 from two independent experiments). Supernatant aliquot of 0.4 ml was neutralized with 0.6 ml of 0.5 M phosphate buffer (pH7.5). For GSSG assay, the GSH was masked by adding 20 µl of 2-vinylpyridine of the neutralized supernatant, whereas 20 µl of water was added in the aliquots utilized for the total glutathione pool (GSH+GSSG) assay. Tubes were mixed until an emulsion was formed. Glutathione content was measured using 1 ml of reaction mixture containing 0.2 mM NADPH, 100 mM phosphate buffer (pH7.5), 5 mM EDTA, 0.6 mM 5,5'dithiobis (2-nitrobenzoic acid) and 0.1 ml of sample obtained as described above. The reaction was started by adding 3 u GR and was monitored by measuring the change in absorbance at 412 nm for 1 min. The amount of GSH was estimated by the differences between the amount of total glutathione and that of GSSG. A standard curve for GSH in the range of 0 - 30 $\mu molml^{-1}$ was prepared for the calculation.

Extraction and analysis of ascorbate

Leaf samples were homogenized in 5% metaphosphoric acid. The homogenate was centrifuged at 18000 × g and the supernatant was then used. The ASC and DHA content were determined based on the methods developed by Kampfenkel et al. (1995) and Foyer et al. (1995) with some modifications.

Statistical analysis

All results reported here are the means of replicates. Data were subjected to the analysis of variance (ANOVA) using STATGRAPHICS plus 5.1 statistical software (Statistical Graphics Corp., Princeton, NJ).

RESULTS

Identification of chilling-tolerant rice cultivars at different growth stages

Forty two rice cultivars were screened for the sensitivity or tolerance to chilling in the light at the bud, seedling and booting stages (Table 1). The livability of buds under 4°C after 5 d, the livability of seedlings under 8°C after 2 d and the seed-setting rate under 15°C after 5 d were used as indexes of chilling tolerance, respectively. The results showed that the identification at the bud or seedling stage was consistent with that at the booting stage. The chilling identification at the booting stage appeared to be related with those of the bud (R^2 = 0.7980) and seedling stage (R^2 = 0.8873) (Figure 1). Similar degree of performance to chilling was observed for different rice varieties at different stages. Furthermore, a plot of chilling tolerance index at different stages showed (Figure 2) that these 42 rice cultivars may be divided into different groups:

(1) J*aponica* cultivars tolerant to chilling temperature: The members of this group include Taipei309, Kitaake and Wuuyujing 3.
(2) *Indica* cultivars sensitive to chilling temperature: The members of this group include CA212, Xiangxian and Pusa.
(3) Hybrid rice cultivars tolerant to chilling at middle degree: The members of this group include Liangyoupeijiu and Shanyou 63.

Japonica hybrid rice cultivars were usually more tolerant to chilling than *indica* hybrid rice cultivars. Therefore, two tolerant cultivars such as Wuyujing 3 and Taipei309, two sensitive cultivars such as CA212 and Pusa and two middle tolerant ones such as Shanyou63 and Liangyoupeijiu were chosen for further examine the physiological and biochemical factors for their responses to the chilling temperature in the light.

Photosynthetic characteristics and chilling tolerance in the light

The curve of photosynthetic rate to PAR may reflect the ability of plants to make use of light energy. In Figure 3, six types of chilling tolerance experiments showed different performance in photosynthesis at various light intensities. Table 2 shows the changes of apparent quantum yield of rice varieties before or after low temperature treatment in the light. It demonstrated that the apparent

Table 1. The chilling tolerance identification of different rice cultivars at the bud stage, seedling stage and booting stage.

Cultivar	Livability under 4℃for 5d at bud stage (%)	Grade of tolerance to chillig	Livability under 8℃ for 2d at seedling stage (%)	Grade of tolerance to chillng	Seed-setting rate after treatment during booting stage (%)	Grade of tolerance to chillin g
pepc transgenic rice(J)	91.13 ± 2.76^{Mkl}	1	84.59 ±3.68^{Rs}	1	83.92 ± 3.25^{Lm}	1
Taipei309(J)	92.95 ± 3.25^{Ml}	1	79.17± 4.33^{QRqrs}	1	85.33 ± 2.76^{Lm}	1
Kitaake(J)	90.41 ± 8.21^{Mkl}	1	81.86 ±5.44^{Rrs}	1	80.11± 3.23^{KLlm}	1
wuyujing3(J)	82.76 ± 7.11^{Mkl}	3	79.67± 5.33^{QRrs}	2	75.58 ± 0.26^{IJKLklm}	2
9516(J)	83.44 ± 6.44^{Mkl}	3	78.31± 3.97^{PQRqrs}	2	76.22 ± 3.56^{IJKLklm}	2
Suhuxiangjing(J)	81.28 ± 7.12^{LMkl}	3	77.97± 4.66^{PQRqrs}	2	75.1 ± 4.28^{HIJKLlm}	2
H45(J)	80.18 ± 6.48^{KLMjkl}	3	74.45 ± 3.33^{NOPQRopqrs}	2	73.3 ± 0.21^{HIJKLijklm}	2
H137(J)	81.31 ± 4.77^{JKLMjkl}	3	73.51 ± 4.77^{NOPQRnopqrs}	2	75.9 ± 4.29^{IJKLklm}	2
9908(J)	84.76 ± 5.33^{Mkl}	3	76.58 ±3.89^{PQRpqr}	2	78.55 ± 1.76^{JKLlm}	2
E32(J)	82.54 ± 4.98^{Mkl}	3	77.16 ±4.27^{PQRqrs}	2	77.58 ±1.89^{JKLklm}	2
Yangfujing8(J)	83.98 ± 5.76^{Mkl}	3	79.12 ±3.83^{QRqrs}	2	75.33 ±2.98^{IJKLjklm}	2
Nanjing44(J)	80.67 ± 7.11^{LMkl}	3	75.56 ± 4.55^{OPQRpqrs}	2	74.99± 2.92^{HIJKLjklm}	2
Huaidao10(J)	83.11 ± 6.33^{Mkl}	3	79.12 ± 3.17^{QRqrs}	2	77.89 ± 2.94^{JKLlm}	2
SZ601(J)	80.27 ± 5.98^{KLMkl}	3	68.88 ± 3.96^{MNOPQmnopq}	3	73.3 ± 0.21^{HIJKLijklm}	2
SZ602(J)	84.12 ± 7.21^{Mkl}	3	63.17 ± 2.97^{LMNlmn}	3	69.45 ±1.42^{GHIJKhijkl}	3
SZ623(J)	84.65 ± 6.45^{Mkl}	3	66.68 ± 3.44^{LMNOPlmnop}	3	62.69 ± 2.98^{EFGHIefghi}	3
SZ624(J)	79.10 ± 6.79^{JKLMjkl}	3	64.45 ± 3.55^{LMNOlmno}	3	61.39 ± 3.67^{DEFGHefghi}	3
SZ617(J)	78.93 ± 6.76^{JKLMjkl}	5	61.73 ± 4.21^{LMlm}	3	65.47 ± 4.21^{FGHIJghij}	3
SZ613(J)	77.58 ± 6.54^{JKLMijkl}	5	57.14 ± 3.82^{KLkl}	4	65.35 ± 2.19^{FGHIJghijk}	3
Shanyou63(I)	76.19 ± 6.08^{IJKLMhijk}	5	49.71 ± 3.21^{JKjk}	5	63.18 ± 2.12^{EFGHIfghijk}	3
Liangyoupeijiu(I)	64.28 ± 5.67^{HIJKLghij}	5	44.81 ± 2.97^{IJij}	5	60.90 ± 4.24^{CDEFdefg}	3
X07s/Zihui100(I)	62.31 ± 4.40^{HIJKfghi}	5	33.61 ± 3.22^{GHIfgh}	6	56.01 ± 3.13^{CDEFGdefg}	4
Yueyou938(I)	61.33 ± 5.29^{HIJfghi}	5	34.70 ± 3.43^{GHIfghi}	6	53.65 ± 4.32^{DEFcdefg}	4
SZ629(I)	59.31 ± 4.39^{HIJfgh}	5	35.87 ± 2.34^{GHIghi}	7	52.65 ± 1.87^{BCDEFcdef}	4
SZ630(I)	55.96 ± 4.22^{GHfg}	5	38.83 ± 2.54^{HIJhi}	7	55.79 ± 2.38^{BCDEFGdefg}	4
SZ631(I)	53.24 ± 4.88^{FGHefg}	5	24.79 ± 2.01^{DEFGdef}	7	50.66 ± 3.64^{ABCDEbcde}	4
SZ632(I)	54.39 ± 4.45^{DEFGHefg}	5	25.34 ± 1.67^{DEFGdef}	7	53.97 ± 2.26^{CDEFcdefg}	4
SZ633(I)	47.32 ± 2.67^{EFGHefg}	7	21.67± 1.89^{DEFde}	7	57.67 ± 3.91^{DEFGdefgh}	4
SZ634(I)	42.88 ± 3.76^{CDEFGHdef}	7	27.34 ± 2.12^{EFGHefg}	7	54.87 ± 2.89^{CDEFcdefg}	4
SZ627(I)	40.23 ± 3.56^{BCDEFbcde}	7	28.07± 1.53^{FGHefg}	7	50.99 ± 3.78^{ABCDEbcdef}	4
SZ635(I)	36.51± 2.87^{BCDEbcd}	7	16.4 ± 1.17^{BCDEcd}	8	54.91 ± 3.25^{CDEFcdefg}	4
SZ636(I)	35.21± 2.99^{BCDbcd}	7	19.89 ±1.32^{DEFde}	8	58.34 ± 4.76^{ABCDEFbcdef}	4
SZ637(I)	30.91 ± 2.87^{BCbc}	7	16.45 ± 1.16^{BCDEFcd}	8	51.54 ± 2.98^{ABCDEbcdef}	4
SZ638(I)	31.92 ± 3.09^{BCbcd}	7	18.77 ± 1.19^{CDEFde}	8	56.9 ± 6.92^{DEFGdefg}	4
SZ639(I)	25.23 ± 0.22^{Bb}	7	15.39 ± 1.03^{BCDbcd}	8	55.34 ± 5.12^{CDEFdefg}	4
X07S(I)	29.11± 0.29^{Bbc}	7	16.39 ± 1.21^{BCDEcd}	8	51.87 ± 3.32^{ABCDEFbcdef}	4
Peiai64S(I)	0^{Aa}	7	19.88 ± 0.87^{DEFde}	7	53.30 ± 3.14^{BCDEFcdefg}	4
IR64(I)	0^{Aa}	9	2.84 ± 0.19^{Aa}	9	43.03 ± 5.32^{ABCabc}	5
Yangdao 6(I)	0^{Aa}	9	3.56 ± 0.21^{Aa}	9	48.35 ± 3.35^{ABCDabcd}	5
Pusa(I)	0^{Aa}	9	7.74 ± 0.23^{ABCabc}	9	40.36 ± 3.69^{ABab}	5
Xiangxian(I)	0^{Aa}	9	5.98 ± 0.21^{ABab}	9	40.12 ± 5.96^{ABab}	5
CA212(I)	0^{Aa}	9	7.17 ± 0.31^{ABCabc}	9	38.25 ± 2.69^{Aa}	5

Evaluation on chilling tolerance at the bud stage in rice were classified at 5 grade as follows: 1 grade = livability at 100%; 3 grade = livability at 80 - 90%; 5 grade = livability at 50 - 79%; 7 grade = livability at 1 - 49%; 9 grade = livability at 0%.
Evaluation on chilling tolerance at seedling stage in rice were classified at 9 grade as follows: 1 grade = seedling livability at 0 - 80%; 2 grade = seedling livability at 70 - 79%; 3 grade = seedling livability at 60 - 69%; 4 grade = seedling livability at 50 - 59%; 5 grade = seedling livability at 40 - 49%; 6 grade = seedling livability at 30 - 39%; 7 grade = seedling livability at 20 - 29%; 8 grade = seedling livability at 10 - 19%; 9 grade = seedling livability at 0 - 9%.
Evaluation on chilling tolerance at booting stages in rice were classified at 6 grade as follows: 1 grade = seed setting rate at 80 - 100%; 2 grade = seed setting rate at 70 - 79%; 3 grade = seed setting rate at 60 - 69%; 4 grade = seed setting rate at 50 - 59%; 5 grade = seed setting rate at 1 - 49%; 6 grade = seed setting rate at 0%.
Capital letters expressed significant at 0.01 level, Lowercase letters expressed significant at 0.05 level.

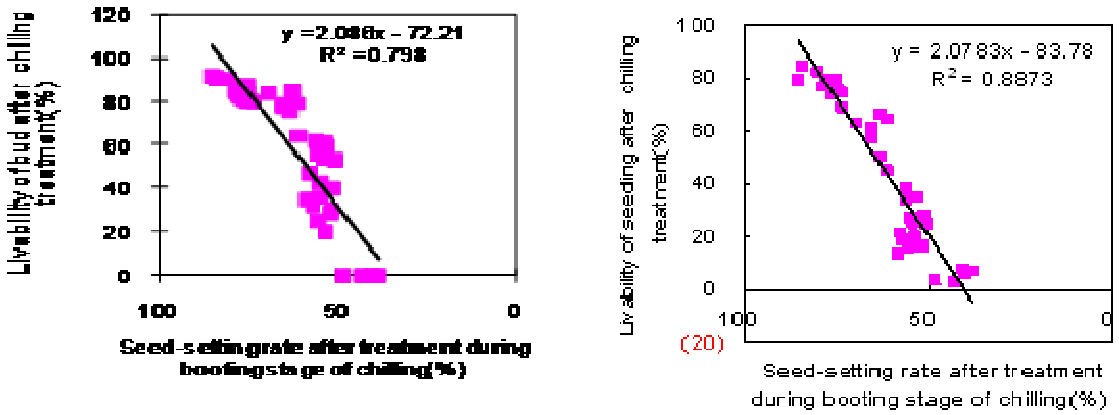

Figure 1. The correlation between seed-setting rate and livability rate of bud and seedling after chilling treatment for rice cultivars.

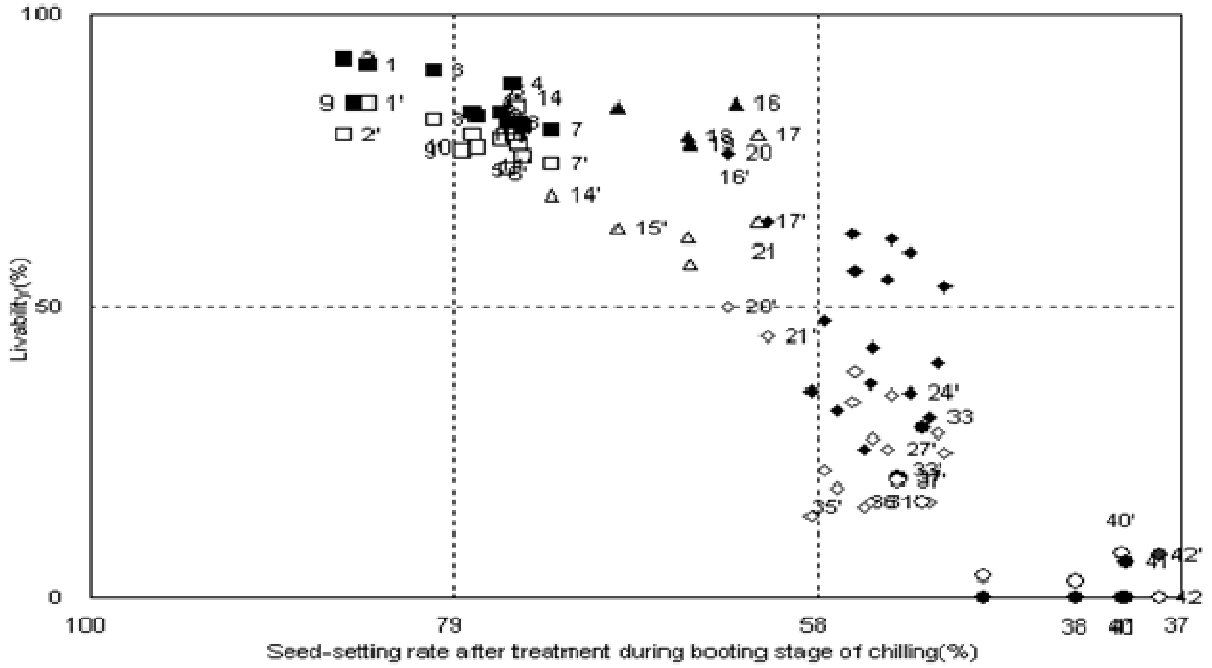

Figure 2. Effect of chilling treatment on livablity rate of bud, seedling and seed-setting rate in 42 rice cultivars at bud, seedling and booting stages. ■ express japonica rice in chilling-identification at bud stage; □ express japonica rice in chilling-identification at seedling stage;▲express japonica hybrid rice in chilling-identification at bud stage; express japonica hybrid rice in chilling-identification at seedling stage; express indica hybrid rice in chilling-identification at bud stage; express indica hybrid rice in chilling-identification at seedling stage; ● express indica rice in chilling-identification at bud stage; ○ express indica rice in chilling-identification at seedling stage. 1 pepc transgenic rice(J)-bud; 2 Taipei309(J)-bud; 3 Kitaake(J)0-bud; 4 wuyujing3(J)-bud; 5 9516(J)-bud; 6 Suhuxiangjing(J)-bud; 7 H45(J)-bud; 8 H137(J)-bud; 9 9908(J)-bud; 10 E32(J)-bud; 11 Yangfujing8(J)-bud; 12 Nanjing44(J)-bud; 13 Huaidao10(J)-bud; 14 SZ601(J)-bud; 15 SZ602(J)-bud; 16 SZ623(J)-bud; 17 SZ624(J)-bud; 18 SZ617(J)-bud; 19 SZ613(J)-bud; 20 Shanyou63(I)-bud; 21 Liangyoupeijiu(I)-bud22 X07s/Zihui100(I)-bud; 23 Yueyou938(I)-bud; 24 SZ629(I)-bud; 25 SZ630(I)-bud; 26 SZ631(I)-bud; 27 SZ632(I)-bud; 28 SZ633(I)-bud; 29 SZ634(I)-bud; 30 SZ627(I)-bud; 31 SZ635(I)-bud; 31 SZ636(I)-bud; 33 SZ637(I)-bud; 34 SZ638(I)-bud; 35 SZ639(I)-bud; 36 X07S(I)-bud; 37 Pei'ai64S(I)-bud; 38 IR64(I)-bud; 39 Yangdao 6(I)-bud; 40 Pusa(I)-bud; 41 Xiangxian(I)-bud; 42 CA212(I)-bud; 1' pepc transgenic rice(J)-seedling; 2' Taipei309(J)-seedling; 3' Kitaake(J)-seedling; 4' wuyujing3(J)-seedling; 5' 9516(J)-seedling; 6' Suhuxiangjing(J)-seedling; 7' H45(J)-seedling; 8' H137(J)-seedling; 9' 9908(J)-seedling; 10' E32(J)-seedling; 11' Yangfujing8(J)-seedling; 12' Nanjing44(J)-seedling; 13' Huaidao10(J)-seedling; 14' SZ601(J)-seedling; 15' SZ602(J)-seedling; 16' SZ623(J)-seedling; 17' SZ624(J)-seedling; 18' SZ617(J)-seedling; 19' SZ613(J)-seedling; 20' Shanyou63(I)-seedling; 21' Liangyoupeijiu(I)-seedling; 22' X07s/Zihui100(I)-seedling; 23' Yueyou938(I)-seedling; 24' SZ629(I)-seedling; 25' SZ630(I)-seedling; 26' SZ631(I)-seedling; 27' SZ632(I)-seedling; 28' SZ633(I)-seedling; 29' SZ634(I)-seedling; 30' SZ627(I)-seedling; 31' SZ635(I)-seedling; 32' SZ636(I)-seedling; 33' SZ637(I)-seedling; 34' SZ638(I)-seedling; 35' SZ639(I)-seedling; 36' X07S(I)-seedling; 37' Pei'ai64S(I)-seedling; 38' IR64(I)-seedling; 39' Yangdao 6(I)-seedling; 40' Pusa(I)-seedling; 41' Xiangxian(I)-seedling; 42' CA212(I)-seedling.

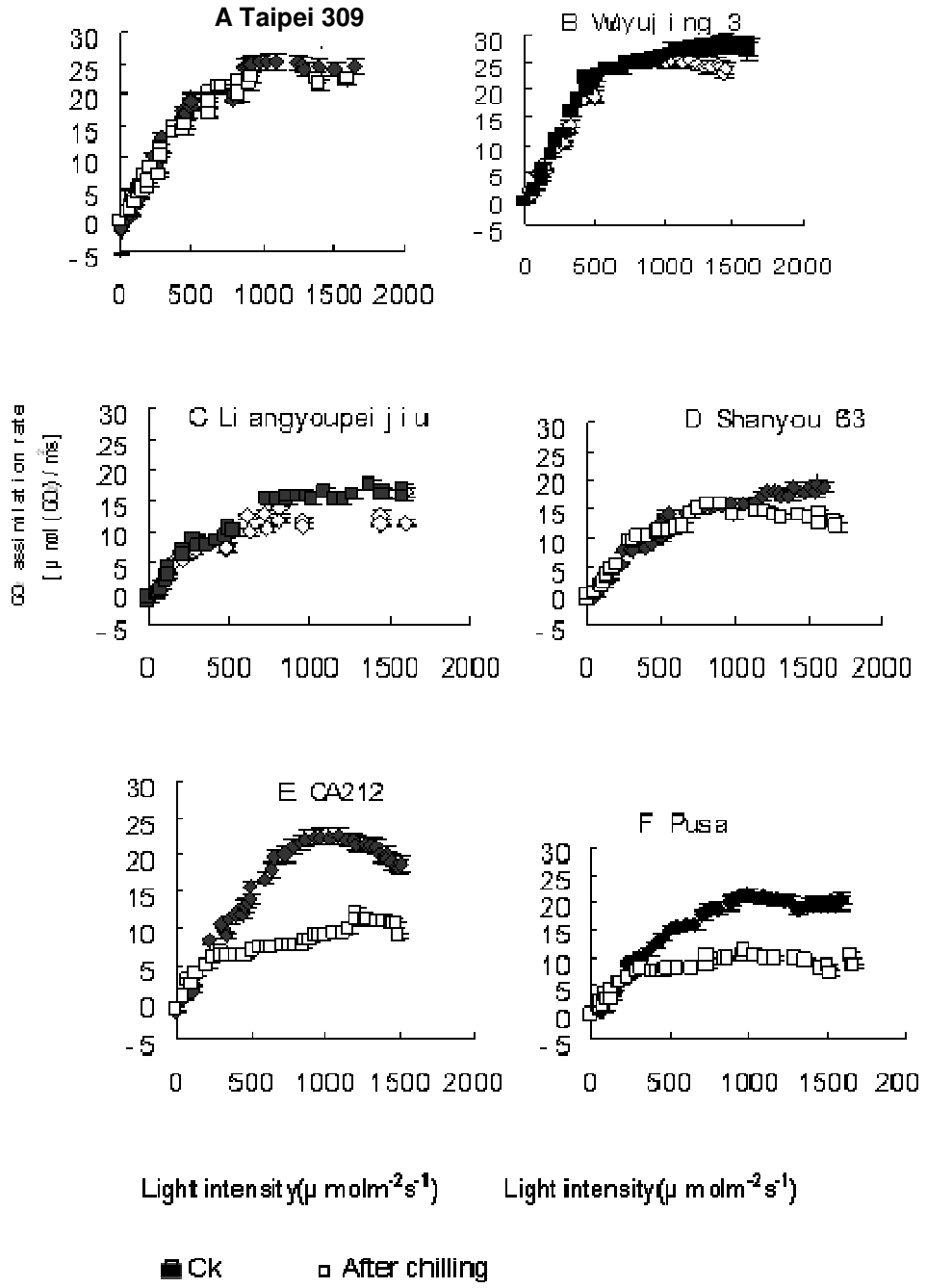

Figure 3. Changes in photosynthetic rate to PAR of leaves of six rice varieties due to chilling treatment.

Table 2. Apparent quantum yield and carboxylation efficiency of six rice cultivars after treatment of low temperature in the light. Measurements were on the top two leaves. Means ± SE (n = 6). Photosynthetic rates of attached leaves were measured at 25°C, 340 μmol/mol CO_2 and 21% O_2. Photosynthetic rates of attached leaves were measured at 25°C, PFD 600 μmol/m^2s and 21% O_2.

Culvitars	Condition	Wuyujing3	Taipei309	Shanyou 63	Liangyoupeijiu	Pusa	CA212
Carboxylation efficiency(mol/mol)	Control	0.0129	0.0158	0.021	0.0538	0.0151	0.0119
	Chilling	0.0205	0.0185	0.0209	0.0216	0.0092	0.0159
Apparent quantum yield (mol CO_2/mol photons)	Control	0.0123	0.0112	0.0129	0.011	0.0118	0.0121
	Chilling	0.0114	0.0111	0.0058	0.0077	0.0076	0.0112

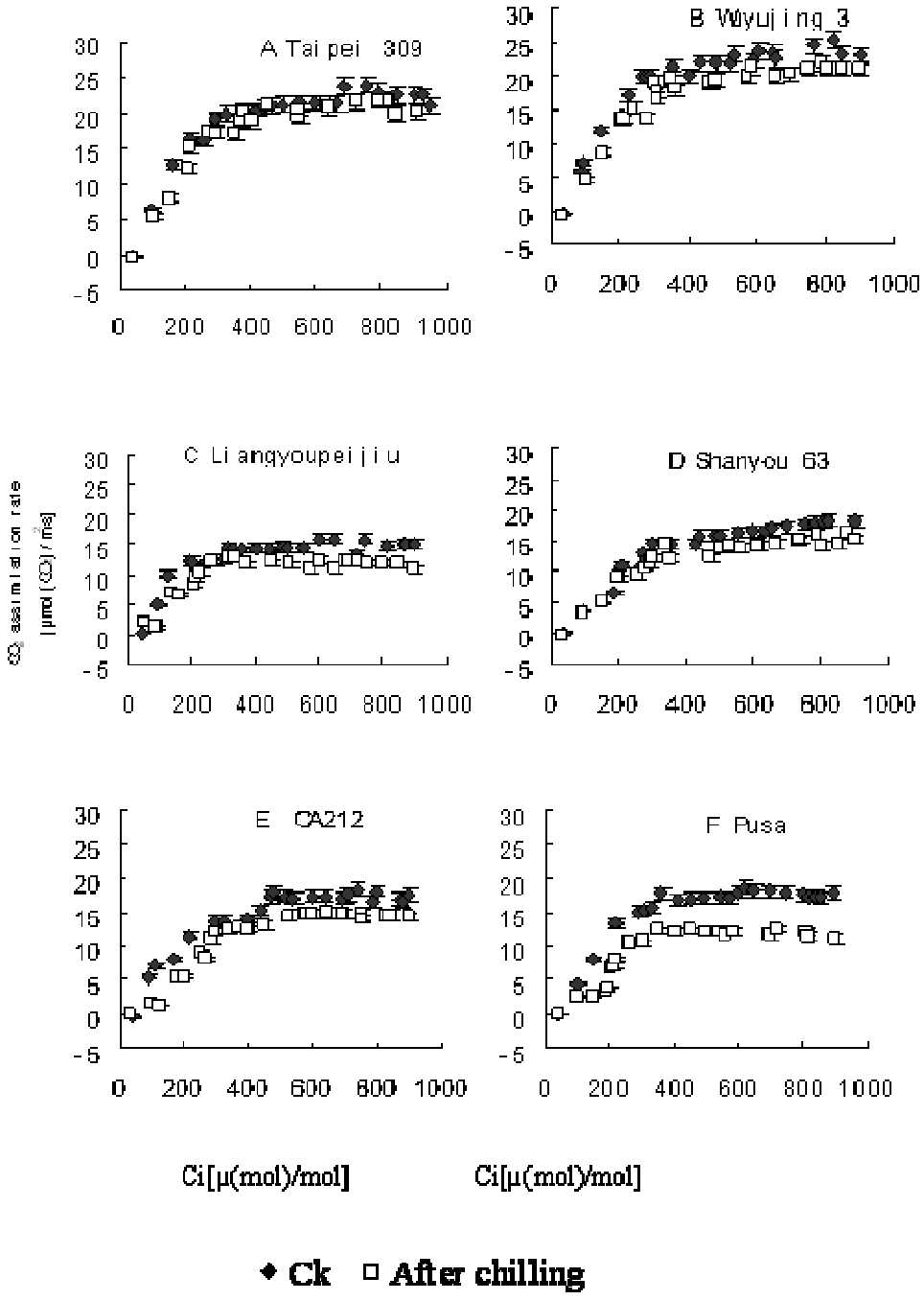

Figure 4. Changes in photosynthesis - CO_2 response curves in the leaves of different rice varieties after chilling treatment

quantum yields of Wuyujing3, Taipei309 and CA212 decreased less than those of Shanyou 63, Pusa and Liangyoupeijiu. At middle and high light intensities (> 600 $\mu molm^{-2}s^{-1}$), obvious changes of photosynthetic rate were observed on the leaves of six rice varieties after low temperature treatment. Compared to the plants without chilling treatment, the tolerant cultivars Wuyujing 3 and Taipei 309 managed to retain higher activities than the sensitive cultivars CA212 and Pusa. It appeared that the sensitive cultivars decreased in both Pm and apparent quantum yield at a low light intensity, resulting in a greater inhibition of photosynthetic rate under high light intensity after chilling treatment than the tolerant ones. These results are consistent with those obtained in the initial screening (Table 1).

At certain CO_2 concentration, the photosynthetic rates of plant increased due to the increase of CO_2 concentration. Figure 4 shows the relationship of photosynthesis

to CO_2 response in the leaves of different rice varieties after chilling treatment. Compared with photosynthesis to light response curves, the photosynthesis to CO_2 response curves of different rice varieties after chilling treatment changed less. The carboxylation efficiency of Wuyujing3, Taipei309, Shanyou63, Liangyoupeijiu, Pusa and CA212 after chilling treatment were respectively 89.50, 98.48, 96.30; 90.80, 60.90 and 91.6% of the corresponding cultivars without chilling treatment (Table 2). The results demonstrated that the Rubisco carboxylation ability to CO_2 were relatively stable and not easy to be changed under chilling condition.

Changes of Chl fluorescence parameters, chlorophyll content, O_2^- forming rates and H_2O_2 contents in six rice cultivars after low temperature treatment in the light

The Chl fluorescence parameters are good indicators for the assessment of PS2 physiological state. As shown in Figure 5, primary photochemical efficiency of PS2 (Fv/Fm) in various rice cultivars decreased to different extent after low temperature treatment in the light as compared to those without treatment. The decreases of Fv/Fm of *japonica* Taipei309 and Wuyujing3 were less than those of *indica* Pusa and CA212, while those of *Indica* hybrid Shanyou 63 and Liangyoupeijiu were between them. Similar to Fv/Fm, low temperature treatment reduced Chl content of cultivars that were susceptible to chilling temperature, such as CA212 and Pusa, more significantly than the tolerant ones, while the *indica* hybrid Shanyou63 and Liangyoupeijiu was between the two different types. As shown in Figure 6, the changes in Chl content and Fv/Fm in leaves of the six rice varieties were consistent. As showing in Figure 5, O_2^- forming rates, MDA content and H_2O_2 content in the cultivars susceptible to chilling temperature accumulated more than those of the tolerant ones, demonstrating the chilling damages. These results demonstrated that the stable PS2 activity (Fv/Fm) after chilling treatment in tolerant rice such as *japonica* subspecies provided the physiological basis to protect photosynthetic rate from the influence of chilling damage.

Changes of fatty acids in six rice cultivars after low temperature treatment in the light

To better understand the sensitivity to chilling, we investigated the content of fatty acids in leaves in six rice cultivars (Figure 7). The content of unsaturated fatty acid (UFA) in the leaves of tolerant cultivars such as Wuyujing3 and Taipei309 were generally greater and the indexes of unsaturated fatty acid (IUFA) were higher than the cultivars susceptible to chilling temperature such as Pusa and CA212 at normal conditions. After low temperature treatment in the light, (the index of Saturated fatty acid) ISFA and IUFA in the tolerant cultivars decreased by 27.1 and 7.1%, respectively, as compared to 34.2 and 9.9% for those of the susceptible cultivars. These results suggested that the difference in fatty acid contents and the changes under chilling temperature might be one of structure basis for the tolerance of chilling temperature in rice.

Changes in the antioxidant content of the different rice varieties after low temperature treatment

Our results showed that, after the low temperature treatment in the light, total glutathione and ascorbate contents in leaves of the six rice varieties were generally enhanced, but different types of chilling tolerance demonstrated different degree of increase (Figure 8). For example, the total ascorbate contents of Wuyujing3, Taipei309, Liangyoupeijiu, Shanyou63, Pusa and CA212 under chilling temperature were increased by 106.0,102.0, 210.0, 136.0, 250.0 and 192.7%, respectively, while the total glutathione contents in these varieties were elevated by 115.7,110.0,128.8, 136.4, 259.0 and 195.7%, respectively. However, it appeared that there was no difference in the total antioxidant content during chilling temperature for these varieties. Further analysis of the glutathione disulphide (GSSG) in glutathione pool after chilling temperature (Figure 8) indicated that GSSG contents in the cultivars susceptible to chilling temperature such as Pusa and CA212 increased by 1226.0 and 928.0%, while GSSG contents in the tolerant cultivars increased only by 140.0 and 118.0%. The changes of dehydroascorbate (DHA) content in the rice cultivars were consistent with the changes of GSSG. Furthermore, the changes of ASA/DHA ratio and GSH/GSSG ratio in the rice cultivars were different for different types; the sensitive cultivars decreased more than the tolerant ones, while the *indica* hybrid rice cultivars were in the middle. It implied that rice's tolerance of chilling in the light might be closely related to the ratio of reduced and oxidative forms of the antioxidant pool, especially to the reduced antioxidant. The role of antioxidant molecular in rice cultivars after chilling temperature would orchestrate different processes through the generation of appropriate signals (H_2O_2) and the balance between oxidant state and reduced state. The Chl content in rice leaves was significantly and negatively correlated with total contents of ascorbate, DHA and H_2O_2 ($p < 0.01$) and significantly and positively correlated with the ratios of ASA/DHA and GSH/GSSG ($p < 0.01$). These results suggested that the ratios of ASA/DHA and GSH/GSSG had greater impact on rice's chilling sensitivity than other indexes examined in this study.

DISCUSSION

The combination of low temperatures and high light inten-

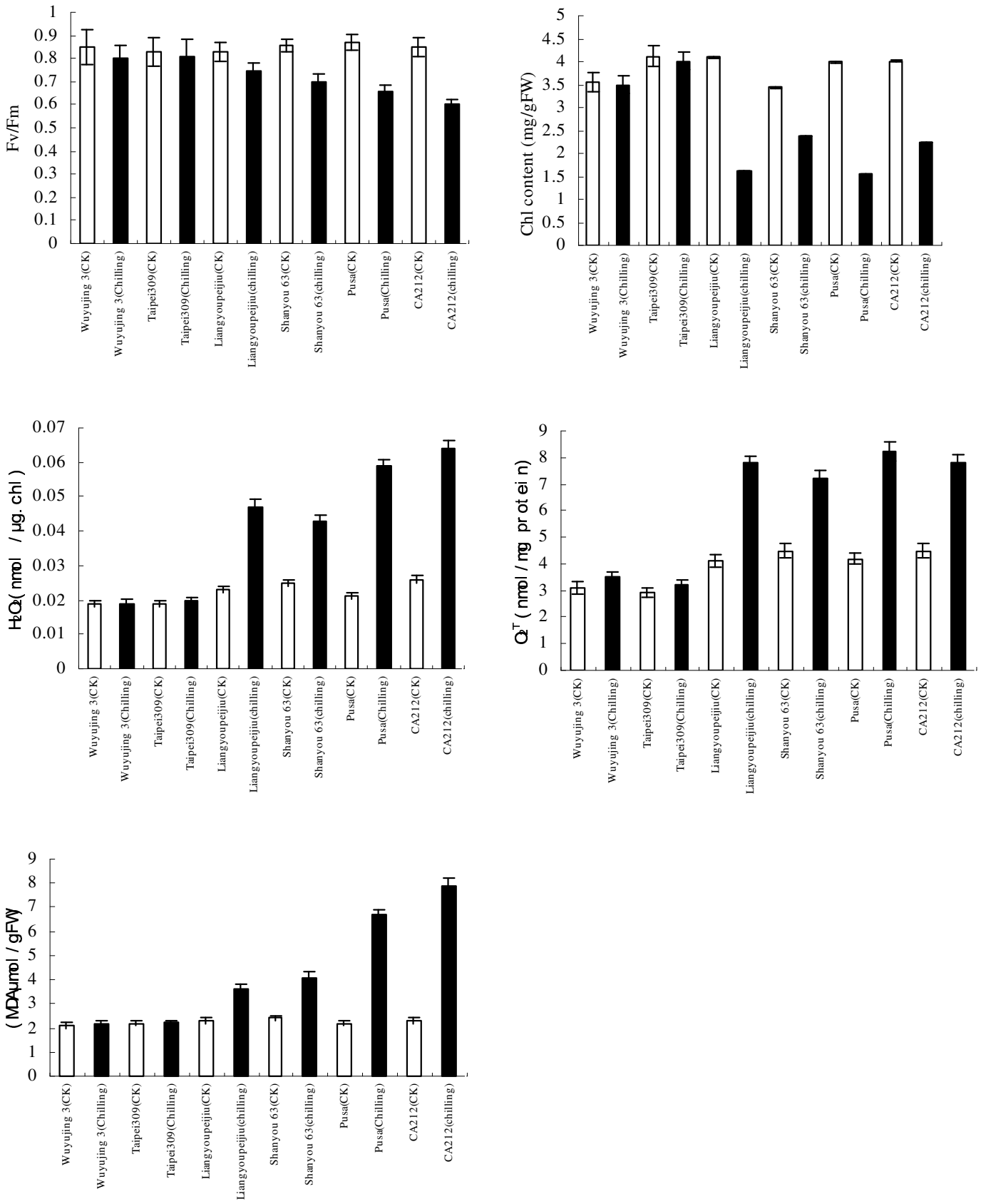

Figure 5. Changes in Fv/Fm, Chl contents, $O_2^{\cdot-}$generation rate, MDA content and H_2O_2 content in leaves of six rice varieties after treatment of low temperature in the light. Measurements were on the top two leaves. Means ± SE (n = 6).

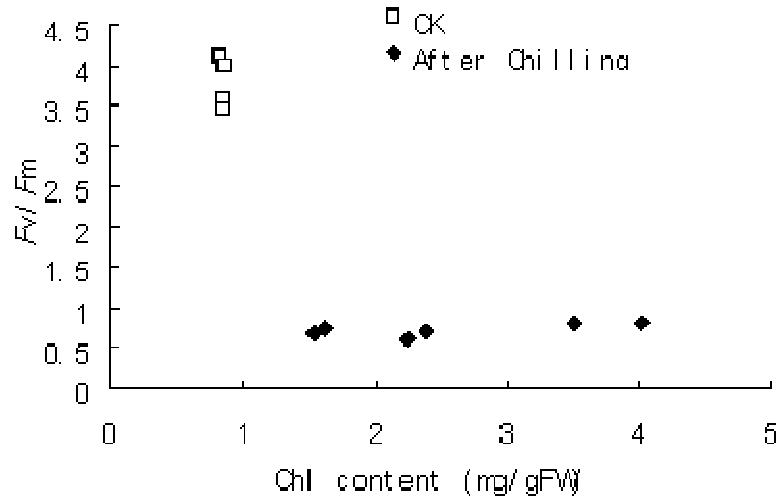

Figure 6. Comparison in chl content and Fv/Fm of leaves of six rice cultivars after treatment of low temperature in the light.

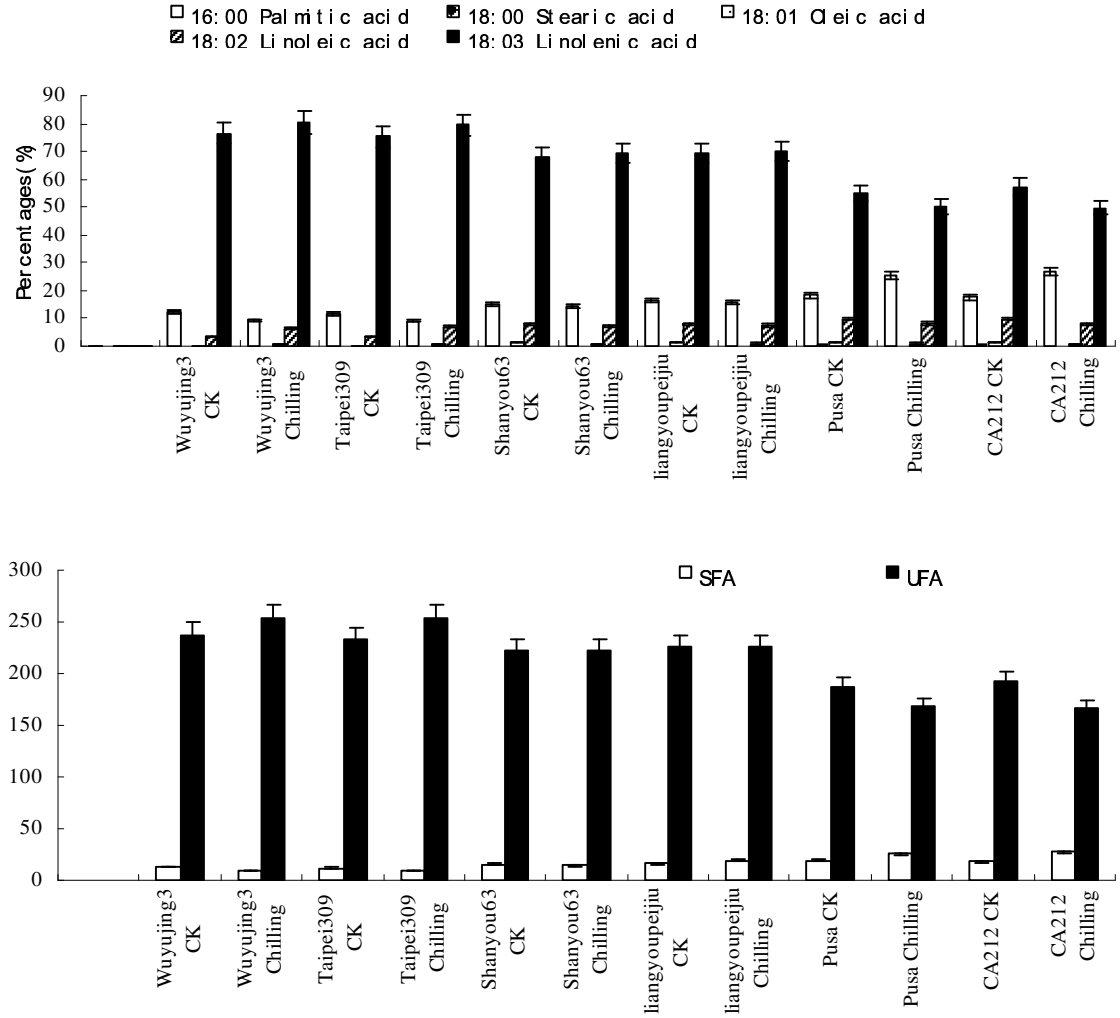

Figure 7. Changes of fatty acid (mol %) of leaves in six rice cultivars. UFA (Index of unsaturated fatty acid) = 18:1+ 18:2 mol%*2+ 18:3 mol%*3. SFA (saturated fatty acid = 16:0 + 18:0 mol%).

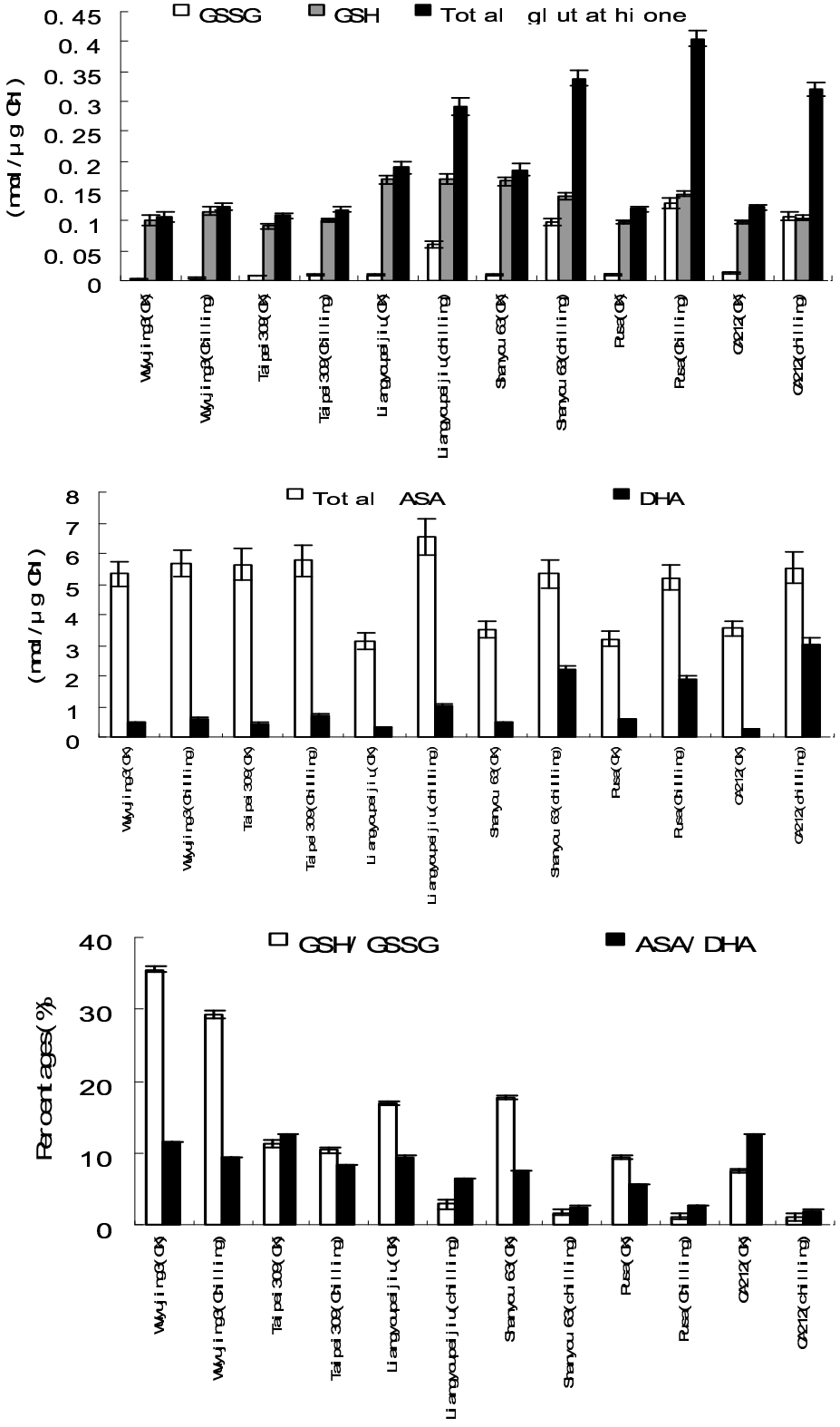

Figure 8. Changes in the antioxidant content (nmol/μg Chl) of six rice cultivars after treatment of low temperature in the light. Measurements were on the top two leaves. Means±SE (n = 6).The correlation coefficients between Chl content and total glutathione,GSSG ,total ASA and DHA were -0.689**, -0.701**, -0.911**, -0.656** and -0.686**, respectively. While those between chl content and ASA/DHA, GSH/GSSG were 0.728** and 0.811**, respectively. ** significant at 0.01 probability level, n = 6.

sity resulted in irreversible inhibition of photosynthesis, mainly due to modifying contents of membrane lipids and the activities of other antioxidant enzymes (Lyons et. al., 1979; Noctor et. al., 2000; Li et. al., 2002b; Viswanathan, 2006). In recent, more researches showed that electron transfer were also the main processing influenced by chilling treatment, which also showing a decrease in the efficiency of light energy conversion in PS2 (Fv/Fm). In this paper, besides these mention above factors, the antioxidants, such as ASA/DHA ratio (r^2 = 0.811) and GSH/GSSG ratio (r^2 = 0.728) also play important role to chilling tolerance in rice. On the whole, we think the mechanism on tolerance of chilling temperature in the light in rice could be explained as follows: when rice plant met with the chilling stress temperature, photosynthesis could be heavily inhibited (Ben, 1987) and more severe even if it was under weak light intensity (Murata, 1989). Light energy conversion in PS2 (Fv/Fm) were influenced at first, However, the motility ability was weak and the protein in the membrane such as PS2-D_1 protein decomposed easily. The activity of VDE (violaxanthinde-epoxidase) and SOD (superoxide dismutase) are thus depressed (Jiao and Ji, 2001). Consequently, the main increase appeared to be GSSG in GSH reductive pool. As GSH that is used for ASA regeneration did not increase, the regeneration of ASA was inhibited and H_2O_2 was not cleaned efficiently. Therefore, the assimilation of O_2^- and H_2O_2 might attack the photosynthetic membranes, resulting in damage to photosynthetic membranes. So we suggested that the antioxidant such as ASA or GSH be another barrier, ahead of modification in membrane lipid composition under chilling treatment in the light.

Rice plants would often meet with chilling stress in the field. Chilling stress not only decreased the ability of photosynthesis but also the yield in rice at late development of stage. So it is very important to breeding rice varieties tolerant to chilling. While chilling identification in rice would be an important first step for evaluation. The previous identification method also focused on observation throughout the whole stage with seed-setting rate as an index of chilling, which was a time-consuming process and screening materials may be limited. In this paper, 42 rice varieties were studied at three different stages in rice. The result showed that the chilling identification at the booting stage appeared to be related with those of the bud (R^2 = 0.7980) and seedling stage (R^2 = 0.8873) (Figure 1), exhibiting that the characteristics of chilling tolerance at earlier developmental stages might find the effect and simple indexes in the future.

The *japonica* rice subspecies was usually more tolerant to low temperature, indicated by a higher regeneration ability of ASA and GSH, a higher index of unsaturated fatty acid under chilling in the light. While the sensitive ones such as *indica* rice subspecies is on the contrary. Those in the *indica* hybrid rice cultivars were in the middle. The strong regeneration ability of ASA and GSH of the tolerant ones such as Wuyujing3 and Taibei309 might be helpful to remove efficiently ROS.

In fact, rice in the process of long-term cultivation has very difference in domesticated rice and wild rice. The different performances tolerant to low-temperature in cultivars were not as typical of wild rice significantly. In this paper, the cultivated rice with middle tolerance of low-temperature might depend more on their regeneration of antioxidants such as ASA or GSH. Therefore, further investigations are needed to determine how GSH/GSSG or ASA/DHA will play the role in cell of cultivars with middle tolerant to chilling.

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REFERENCES

Asada K (2000).The water-water cycle as alternative photon and electron sinks, Philosophical Transctions of the Royal Society of London. Series B. Biol. Sci. 355: 1419-1431.

Aron DI (1949). Copper enzymes in isolated chloroplast: polyphenoloxydase in beta vulgaris, Plant Physiol. 24: 1-15.

Ben GY (1987).Effects of chilling and water stress on photosynthesis of cucumber and pea leaf discs and its relation to light, Acta. Phytophysiol. Sin. 13: 386-394.

Cheng SH, Min SK (2000). Rice varieties in China: status Quo and prospect, China Rice, 1: 13-16.

Cheng SH, Zhai HQ (2000). Comparison of some plant type components in super high-yielding hybrids of intersubspecies rice, Acta Agron. Sin. 26: 713-718.

Conklin PL (2001). Recent advances in the role and biosynthesis of ascorbic acid in plant, Plant Cell Environ. 24: 383-394.

Foyer CH, Souriau N, Perret S (1995). Measurements of the ascorbate content of spinach leaf protoplasts and chloroplasts during illumination, Planta, 157: 239-244.

Genty B, Briantais JM, Baker NR (1989). The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochem. Biophys. Acta, 900: 87-92.

Heath RL, Packer L (1968). Photoperoxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125: 189-198.

Jiao DM, Ji BH (2001).Photoinhibition in *indica* and *japonica* subspecies of rice (*Oryza sativa*) and their reciprocal F_1 hybrids. Aust. J. Plant Physiol. 28: 299-306.

Jiao DM, Ji BH, Li X (2003).Characteristics of chlorophyll fluorescence and membrane-lipid peroxidation during senescence of flag leaf in different cultivars of rice. Photosynthetica, 41: 33-41.

Kampfenke K, Van Montagu M, Inze D (1995). Extraction and determination of ascorbate and dehydroascorbate from plant tissue, Analyt. Biochem. 25: 165-167.

Li X, Jiao DM, Liu YL, Huang XQ (2002a). Chlorophyll fluorescence and membrane lipid peroxidation in the flag leaves of different high yield rice variety at late stages of development under natural condition, Acta Bot. Sin. 44: 413-421.

Li X, Liu YL, Jiao DM (2002b). The relationship between diurnal variation of fluorescence parameters and characteristics of adaptation to light intensity in leaves. Acta Agron. Sin. 28: 145-153.

Li X, Cheng Y (2005). Identification of chilling at different stages in rice. Jiangsu Agric. Sci. pp. 23-26.

Li X, Dai CC, Jiao DM, Foyer CH (2006). Effects of low temperature in

the light on antioxidant contents in rice (*Oryza sativa* L.) Indica and Japonica subspecies seedlings. J. plant physiol. Mol. Biol. 32: 345-353.
Luwe M, Takahama U, Heber U (1993). Role of ascorbate in detoxifying ozone in the apoplast of spinach (*Spinacia oleracea* L.) Leaves, Plant Physiol., 101:969-976.
Lyons JM, Raison IK, Steponkus PL (1979).The plant membrane in response to low temperature: an overview.-Lyons JM. Low Temperature stress in crop plants. New York, Academic Press, pp. 1-24.
Murata N (1989). Molecular species composition of phosphatidylglycerols from chilling-sensitive and chilling-resistant plants. Plant Cell Physiol. 24: 81-86.
Noctor G, Sonja VJ, Foyer CH (2000). Peroxide processing in photosynthesis: antioxidant coupling and redox signaling, Phil. Trans. R. Soc. Lond. B. 355: 1465-1475.
Patterson BD, Mackae EA, Ferguson IB (1984). Estimation of hydrogen peroxide in higher plant. Phytochemistry, 21: 2771-2881.
Viswanathan C, Zhu JH, Zhu JK (2006). Gene regulation during cold acclimation in plants. Physiol. Plant. 126: 52-61.
Wang AG, Luo GH (1990). Quantititive relation between the reaction of hydroxylamine and superoxide anion radicals in plants. Plant Physiol. Commun. 6: 55-57.
Yu HG, Su WA (1996). Studies on relationship between fatty acids desaturation of PSII membrane and low temperature photoinhibition of cucumber, Acta Biophys. Sin. 2: 227-233.