

## Response to High Temperature in Flag Leaves of Super High-Yielding Rice Pei'ai 64S/E32 and Liangyoupeijiu

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**Abstract:** Two newly bred hybrid rice combinations, super high-yielding Liangyoupeijiu (Pei'ai 64S×9311) and Pei'ai 64S/E32 (Pei'ai 64S×E32) were used to investigate the photosynthetic characteristics under high temperature in comparison with hybrid rice Shangyou 63. High temperature caused a decreased photosynthetic efficiency and aggravated photoinhibition. The optimum temperature for photosynthetic electron transportation and photosynthetic CO<sub>2</sub> fixation were about 28°C and 35–40°C respectively. Linear electron transportation is more sensitive to high temperature than the photochemical process. The mechanism of high temperature adaptation was possibly as follows: super high-yielding rice has quickly increasing carotenoid, which acted as a more favorable antioxidant system to reduce the active oxygen production and avoid damage to the photosynthesis system; super high-yielding rice has a higher efficiency of xanthophylls cycle to dissipate excess heat energy; super high-yielding rice has a more stable photosynthetic function, higher photosynthetic efficiency and more heat stable protein content.

**Key words:** hybrid rice; high temperature; stress; photosynthesis

Rice, grown in the field, is always experienced changes in light and temperature. The photosynthetic capacity of rice is directly inhibited by high and low temperature<sup>[1]</sup>. Extremely high temperature is one of the main disasters exist in the rice growing areas all over the world<sup>[2]</sup>. The natural environment in South China always has negative effects on the rice production, and high temperature in summer may result in the abnormal seed setting, such as, large quantities of shrunken grains and lower grain yield caused by unfilling or deficient filling, which bring about yield reduction in large areas<sup>[3, 4]</sup>. The grain filling stage of early season rice in South China is just under high temperature summer conditions. In order to improve the utilization capacities of rice cultivars to light and temperature, ideotype cultivars should have high photosynthetic capacities, and better adaptation to the alternation of light and temperature<sup>[5]</sup>. Series of physiological and biochemical characteristics of rice can be directly affected by high temperature, such as the stability of membrane system, enzyme reaction, the utilization of water and nutrition and the synthesis

of hormones, and so on. A lot of enzymes and membrane protein compounds relate to photosynthesis are distributed on the chloroplast thylakoid membrane, therefore the photosynthetic capacities are directly influenced by the stability of membrane system at high temperatures. To select, breed and apply elite rice cultivars with high resistance to heat is not only the main measures to eliminate the high temperature damage<sup>[4]</sup>, but also the effective method to ensure high and stable product at high temperatures. To study the adaptation characteristics of super high-yielding rice to high temperature can understand the resistance mechanisms of rice with high yield and also be helpful to breed new cultivars with high and stable yield and high temperature resistance. It was reported that there existed difference of resistance to high temperature among rice cultivars<sup>[6]</sup>. However, the photosynthetic mechanisms of the adaptation of rice to high temperature are less concerned. Super high-yielding rice cultivars, bred in recent years, are new combinations with excellent performance. It is of important theoretical significance and practical value to study the photosynthetic mechanism of the adaptation to high temperature of super high-yielding rice.

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## MATERIALS AND METHODS

### Materials

The seeds of super high-yielding rice combinations (*Oryza sativa* L.), Pei'ai 64S/E32 (Pei'ai 64S × E32), Liangyoupeijiu (Pei'ai 64S × 9311) and high-yielding hybrid rice Shanyou 63 as control were supplied by Hunan Hybrid Rice Center. They were sowed on 1 August 2002, and were transplanted to the experimental field of South China Institute of Botany on 25 August with routine managements. The booting stage began from 20 October and the grain filling stage from 5 November. The temperature was between 20°C and 25°C in the day time at the heading stage. Flag leaves were taken at the filling stage, whose middle sections were soaked in the distilled water treated with different temperatures from 36°C to 48°C respectively for 30 min without light. The leaves treated at 28°C were taken as control. All the measurement parameters were got after treatments.

### Methods

#### *Measurement for pigment contents*

Discs of leaves were extracted by 80% acetone. The chlorophyll and carotenoid contents were measured according to the method of Lin et al [7], using UV-Vis spectrophotometer (Lambda 25, Perkin Elmer Ins, USA).

#### *Measurement for photosynthetic oxygen evolution*

The photosynthetic oxygen evolution rate of leaf discs was measured by oxygen electrode (Hansatech, England) at different PPFD with fixed temperature of 25°C and saturate CO<sub>2</sub> concentration. The photosynthetic oxygen evolution-light responses curves were made with the PPFD (μmol / m·s) for *x*-axis and the oxygen evolution rate for *y*-axis, and the net photosynthetic oxygen evolution rate ( $P_n$ ), the maximum oxygen emission rate ( $P_{max}$ ), the apparent photon efficiency ( $\Phi_i$ ), expressed by the ratio of oxygen emission rate and PPFD (the slope of the straight line) under low illumination (0–200 μmol / m<sup>2</sup>·s), were also calculated.

#### *Measurement for chlorophyll a fluorescence*

The chlorophyll a fluorescence induced kinetics

of the rice leaves *in vitro* were measured by pulse modulated fluorescence measurement PAM 101/102/103 fluorometer (Heinz, Walz, Effeltrich, Germany) at the temperature of 25°C. The detached leaves were dark-adapted for 15 min before measurement. The original fluorescence yield ( $F_o$ ) was determined with a low intensity of modulated beam (1.6 kHz, 0.05 μmol / m<sup>2</sup>·s).  $F_m$  was induced by high saturation light (Schott lamp KL 1500 FL 103, 6000 μmol / m<sup>2</sup>·s, 2 s), while the PAM modulated measuring beam (PAM 102) automatically switched to 100 kHz. The  $F_m'$  was determined 15 min after the actinic light (300 μmol / m<sup>2</sup>·s) treatment with a saturating pulse. The maximum PSII quantum efficiency  $F_v/F_m = (F_m - F_o) / F_m$ , the activity of PSII  $F_v/F_o = (F_m - F_o) / F_o$ ,  $q_P$  (photochemical quenching coefficient) and  $q_N$  (non-photochemical quenching coefficient) were calculated according to Schreiber et al [8] and Genty et al [9], and all the chlorophyll parameters were relative values. The possibility of the photoinhibition in photosynthetic apparatus was indicated by  $1 - q_P/q_N$  [10]. When measuring the curve of  $F_o$  response to the temperature, leaf discs were put into the reaction cup covered by black cloth under a water bath. The temperature of water bath increased 2°C/min when the changes of  $F_o$  with temperature were recorded. The temperature at the point of  $F_o$  curve which  $F_o$  was raised suddenly was called  $T_i$  (inflexion temperature) and  $F_o$  was reduced called  $T_p$  (peak temperature).

#### *Measurement of soluble proteins*

According to Bradford [11], measurements of light absorbance were carried out at 595 nm, with Coomassie brilliant blue G-250 stained and the standard curves were made by Albumin Bovine.

#### *Measurement of heat-stable proteins*

According to Fu et al [12], 0.5 g leaves were used for extraction with 5 mL phosphate buffer (50 mmol / L, pH 7.0, with 0.1 mmol / L EDTA, 0.1% Triton X-100). After treatments in boiled water for 15 min, in ice water for 30 min and centrifuged with 10 000 × *g* for 15 min, the supernatant i.e. heat-stable protein solution was obtained. The methods were referred to soluble protein measurements.

*Measurements of the activities of CAT*

The leaves (0.5 g) were taken for extraction with 5 mL phosphate buffer (50 mmol/L, pH 7.0, within 0.1 mmol/L EDTA, 0.1% Triton X-100) and a little quartz sand. After centrifuged at 20 000×g for 15 min, the supernatant was the crude extraction for analysis of the activities of enzyme. Measurement of CAT activities was referred to Zeng et al [13].

*Measurements of the total antioxidant capacity (DPPH· method)*

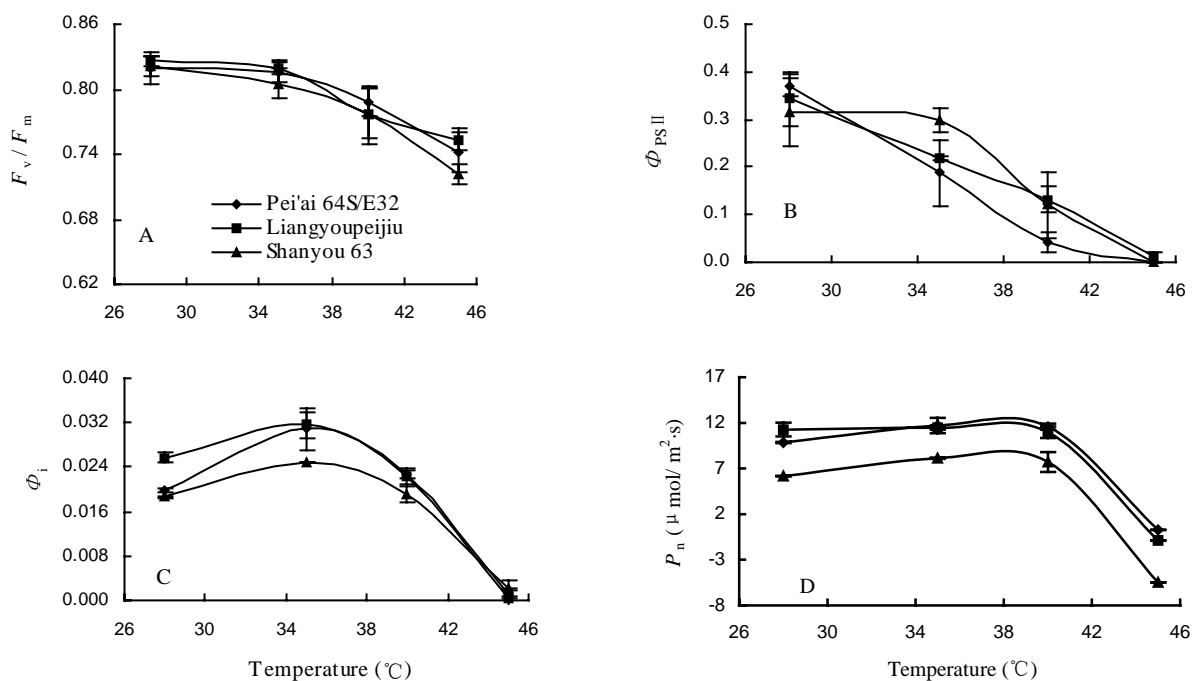
The measurement of the scavenging capacity of DPPH· (1,1-diphenyl-2-picrylhydrazyl) was according to Peng et al [14] with slight modifications. DPPH· solution has a unique purplish red absorption peak. The organic free radical scavenging capacity (ORSC) can be indicated by the reduction of A<sub>525</sub> after adding antioxidant or plant extraction to DPPH· solution. The whole reaction volume was 2 mL, the concentration of DPPH· (dissolved in little carbinol and made by 50% ethanol) was 120 μmol/L. The reaction system contained 0.1 mL plant extraction (made by 50% ethanol) and 1.9 mL DPPH·. A<sub>525</sub> was measured after 20 min's reaction. The remaining DPPH·%,  $R = [(A - B) / A_0] \times 100\%$ , the scavenging ratio (ORSC%) =  $(1 - R) \times 100\%$ . A<sub>0</sub> was the absorption of

DPPH· without plant exaction (1.9 mL DPPH·+0.1 mL 50% ethanol). A was the absorption of DPPH· after reaction with samples. B was the absorption of blank check (0.1 mL sample + 1.9 mL 50% ethanol). The scavenging capacity to DPPH· (μg DPPH·/μg sample) to antioxidant or plant sample was calculated by the formula of (ORSC% × the weight of DPPH· in reaction) / sample weight.

**RESULTS**

**Changes in photosynthetic efficiency in flag leaves of hybrid rice at different high temperatures**

The photochemical efficiency of PSII,  $F_v/F_m$ , and the quantum efficiency of liner electron transportation across PSII,  $\Phi_{PSII}$ , reached the highest at 28°C in all the three combinations (Fig. 1-A, B). Both of  $F_v/F_m$  and  $\Phi_{PSII}$  decreased with temperature. There is little difference in  $F_v/F_m$  between two super-yielding rice combinations and control at 28, 35 and 40°C. The decrease of  $F_v/F_m$  was smaller than that of  $\Phi_{PSII}$ . Compared with 45 °C,  $F_v/F_m$  only declined by 8.8–21.0% at 28°C, while 96.7–100% for  $\Phi_{PSII}$ , which showed that the linear electron transportation was more sensitive to high temperature than the photochemical process. Super high-yielding rice Pei'ai 64S/E32 and Liangyoupeijiu displayed higher



**Fig. 1. Changes in photosynthetic efficiency in flag leaves of hybrid rice at different high temperatures.**

apparent quantum efficiency  $\Phi_i$  and net photosynthetic rate  $P_n$  than Shanyou 63. With the increasing of temperature, both  $\Phi_i$  and  $P_n$  increased at the beginning but dropped later, and reached the highest level at 35°C but declined quickly at 45°C, which suggested that the photosynthetic apparatus was much more stable and the carbon assimilation rate reached the utmost during the temperature of 35–40°C.

### Changes in chlorophyll fluorescence quenching in flag leaves of hybrid rice at different high temperatures

Super high-yielding rice Pei'ai 64S/E32 and Liangyoupeijiu had higher  $q_p$  and lower  $q_N$  at the room temperature 28°C (Fig. 2).  $q_p$  decreased and  $q_N$  increased with the treated temperature. The  $q_N$  of super high-yielding rice Pei'ai 64S/E32 and Liangyoupeijiu displayed higher value at high temperature (35, 40 and 45°C). However, the  $q_N$  of Shanyou 63 increased slowly with treated temperature and was lower at 45°C than at 40°C, which exhibited low  $q_N$  at high temperature.

### Changes in $F_o$ , $T_i$ and $T_p$ in flag leaves of hybrid rice under high temperature treatments

The primary fluorescence  $F_o$  represents the fluorescence emission of the excited antenna chlorophyll a molecules before the transportation of excited energy to reaction center. It only depends on the primary density of excited proton in PSII pigments and state of thylakoid structure, and has no relationship with photochemical process [15]. The membrane structure of thylakoid is sensitive to heat [16], therefore,  $F_o$  can reflect the changes of

membrane structure induced by heat, and it is a good physiological index in studying the plant resistance to heat [17]. Medium stress of heat has a reversible effect on membrane structure and there is no significant change in  $F_o$ . When the stress reaches a definite threshold, the destruction of membrane structure will be irreversible, with the sudden increase of  $F_o$ , which has a close relationship with the functional separation of the PSII reaction center and core antenna pigments [16]. And when the stimulation of heat results in the complete destruction of PSII reaction center in thylakoid,  $F_o$  will start dropping. Both  $T_i$ , the inflexion temperature of  $F_o$ 's sudden increase, and  $T_p$ , the peak temperature of  $F_o$ 's decrease, can be used to evaluate the heat resistance of plant. As shown in Fig. 3, the super high-yielding rice had a higher  $T_i$  and  $T_p$  than high-yielding rice Shanyou 63, which represented better heat resistance characteristics.

### Changes in CAT activity and total antioxidant capacity in flag leaves of hybrid rice at different high temperatures

After treatments of different high temperatures, the CAT activity in flag leaves decreased gradually. Pei'ai 64S/E32 showed the highest CAT activity. The activity of Liangyoupeijiu was a little higher than Shanyou 63 at 28°C and 35°C, but had no remarkable difference at 40°C and 45°C (Fig. 4-A). Scavenging the organic free radical DPPH· is a simple and feasible method to evaluate the total antioxidant capacity of plant [14]. Compared with the room temperature, the total antioxidant capacity was increased in all the three combinations at high temperature treatment, which increased swiftly before 35°C but gradually

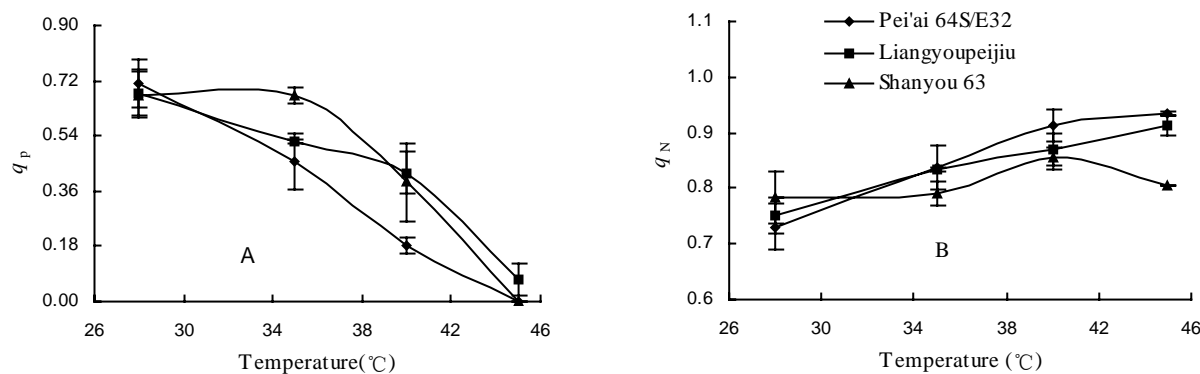


Fig. 2. Changes in chlorophyll fluorescence quenching in flag leaves of hybrid rice at different high temperatures.

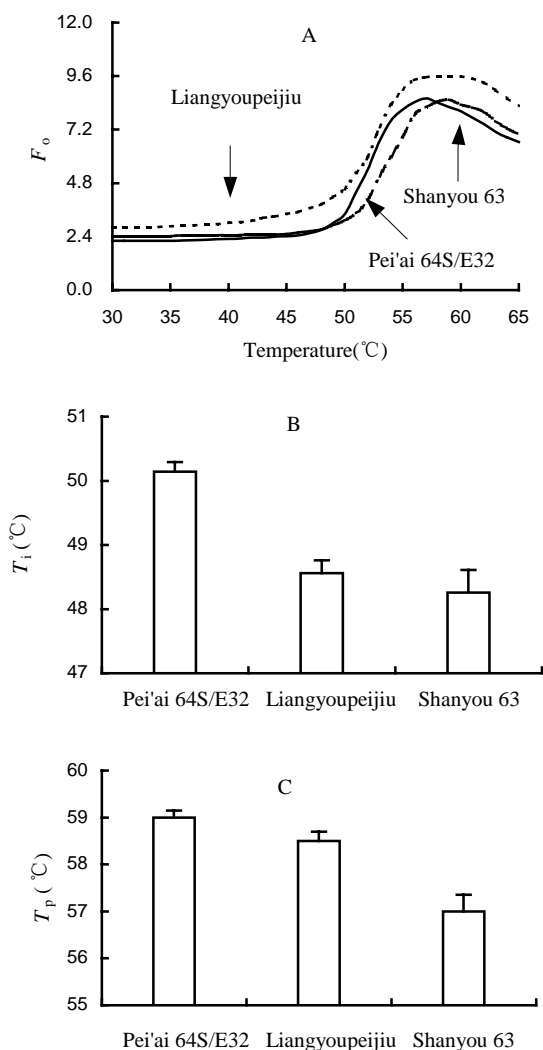


Fig. 3. Changes in F<sub>o</sub> (A), inflexion temperature T<sub>i</sub> (B) and T<sub>p</sub> (C) in flag leaves of super high-yielding hybrid rice and Shanyou 63 under high temperature treatments.

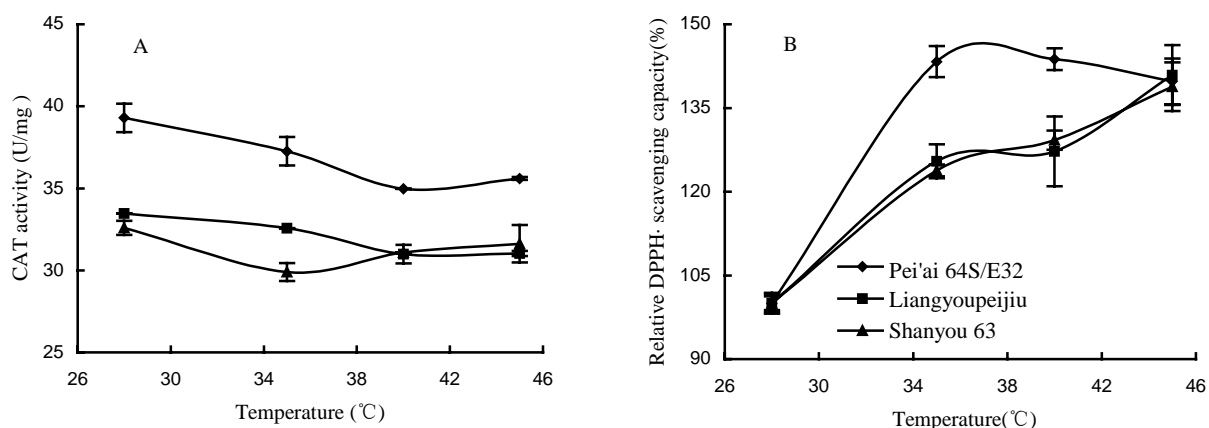


Fig. 4. Changes in CAT (A) activity and total antioxidant capacity (B) in flag leaves of hybrid rice at different high temperatures.

later. Liangyoupeijiu had a similar change with Shanyou 63. The increase of total antioxidant capacity of Pei'ai 64S/E32 was much better than that of Shanyou 63 at 35°C and 40°C (Fig. 4-B).

### Changes in soluble protein and heat stable protein in flag leaves of hybrid rice under different high temperature treatments

The content of the soluble protein increased at the beginning and decreased later with the treatment temperature (Fig. 5-A), and reached the maximum value at 40°C in all the three combinations. Heat stable protein content also increased along with the elevated temperature in Pei'ai 64S/E32, and was higher in super rice Pei'ai 64S/E32 and Liangyoupeijiu than in Shanyou 63. It reached the highest level quickly in Pei'ai 64S/E32 at 35°C and was 1.58 times more than Shanyou 63, but declined at 40 and 45°C. And the content in Liangyoupeijiu and Shanyou 63 rose slowly with the temperature and had their highest value at 35 and 40°C, respectively.

### Changes in Car content and Car/Chl in flag leaves of hybrid rice at different high temperatures

As shown in Fig. 6, Car content in the flag leaves increased in all the three combinations with the temperature. It increased gradually before 35°C, but more quickly in super high-yielding rice Pei'ai 64S/E32 and Liangyoupeijiu than in Shanyou 63 above 35°C. The ratio of Car/Chl rose with the elevated temperatures, and was relatively higher in Liangyoupeijiu and Pei'ai 64S/E32 than in Shanyou

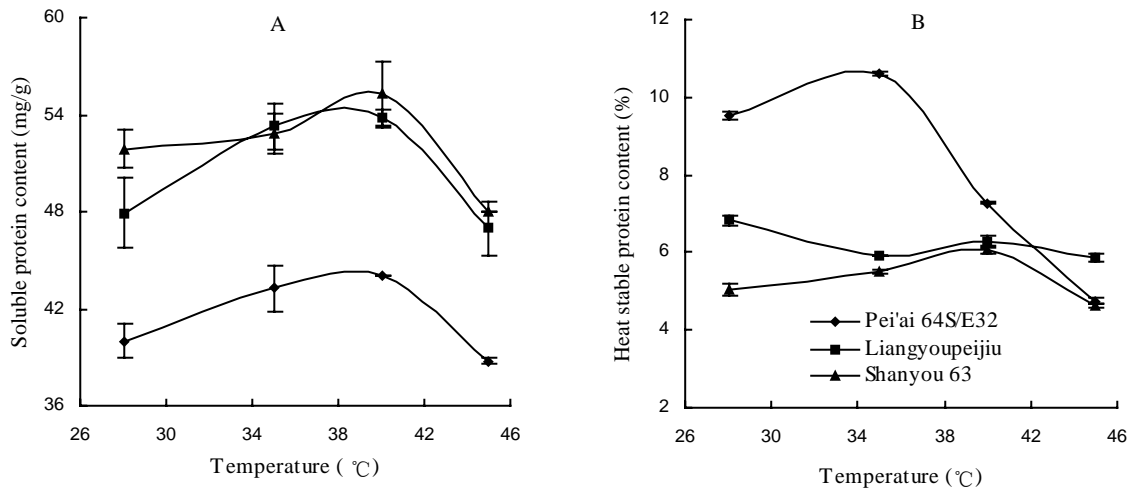


Fig. 5. Changes in soluble protein (A) and heat stable protein (B) in flag leaves of hybrid rice at different high temperatures.

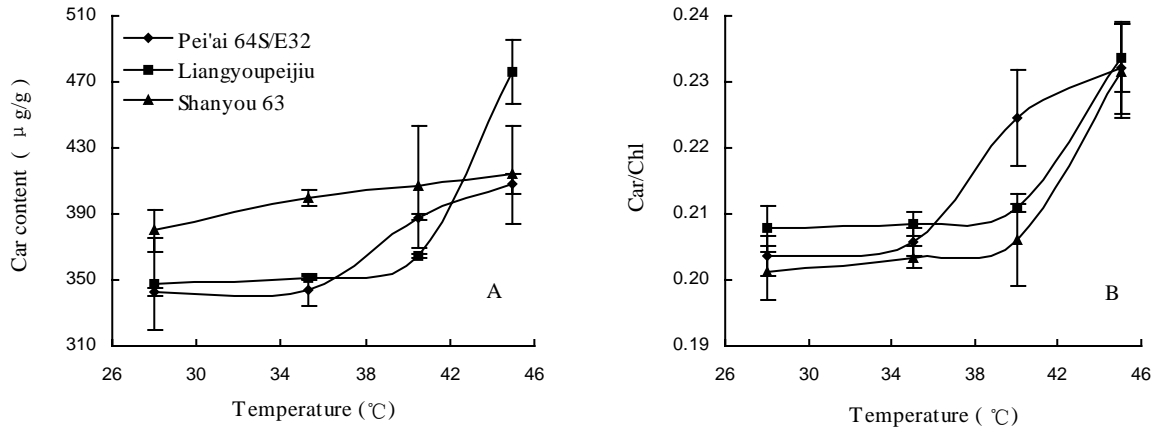


Fig. 6. Changes in Car content (A) and Car/Chl (B) in flag leaves of hybrid rice at different high temperatures.

63 under different temperature treatments.

## DISCUSSION

There existed different responses to high temperature among three hybrid rice combinations including two super high-yielding rice combinations (Pei'ai 64S/E32 and Liangyoupeijiu) and their control Shanyou 63. Two super high-yielding rice combinations displayed higher tolerance to high temperature than control. High temperature above 35°C resulted in decreased photosynthetic efficiency in the three rice materials (Fig. 1), which showed that high temperature aggravated photoinhibition. The optimum temperature was about 28°C for photochemistry and photosynthetic electron transportation (Fig. 1-A, B),

and 35–40°C for carboxylation. Super high-yielding rice had higher  $T_i$  and  $T_p$  after high temperature treatments (Fig. 3), which exhibited more stable photosynthetic system. Compared with  $F_v/F_m$  (decreased by 8.8–21.0%), the decline ratio of  $\Phi_{PSII}$  was high (96.7–100%) at 45°C (Fig. 1), which suggested that the linear electron transportation is more sensitive to high temperature than the photochemical process. Below 40°C, the synthesis of soluble protein and heat stable protein increased with the temperature, in which the increase of heat stable protein only made up a small part of soluble protein. Rubisco constitutes more than half of the soluble protein. The increase of soluble protein was mainly caused by the enhancement on the expression of Rubisco. Because of the improvement of the

expression of Rubisco, active Rubisco was increased and net photosynthetic rate was elevated. In comparison with 40°C, the decrease of soluble protein content may mainly result from the loss of Rubisco at 45°C. Rubisco was deactivated or denatured and the net photosynthetic rate slowed down when leaves were treated with high temperature.

$q_N$  has a direct correlation with the content of zeaxanthin in xanthophylls cycle<sup>[18]</sup>. Even though the molecular mechanism of zeaxanthin is not clear, most results showed that it participated in the non-radiation dissipation process of superfluous excitation energy and played an important role in protecting photosynthetic apparatus against excess light energy<sup>[19]</sup>. The elevation of  $q_N$  showed the increase of non-radiation dissipation by xanthophylls cycle in rice flag leaves. As the temperature rised, more excitation energy was distributed to the non-photochemical way by the increase of non-radiation dissipation. Meanwhile, the decline of  $q_P$  can keep PSII in a higher oxidative state so as to protect photosynthetic apparatus. The super rice had higher  $q_N$  and lower  $q_P$  than Shanyou 63 under high temperature stress (Fig. 2), which showed that the efficient operation of xanthophylls cycle in super rice could reduce the potential danger of excess excitation energy in photosynthetic apparatus. Accumulation of active oxygen was one of the main reasons for plant damage under stresses, which resulted from the unbalance of metabolism of production and elimination active oxygen<sup>[20]</sup>. High temperature can aggravate the photoinhibition<sup>[21]</sup> and affect the distribution and utilization of exciting energy in photosynthetic apparatus. Photosynthetic apparatus can dissipate excess exciting energy by electron transportation in water cycle<sup>[22]</sup>. CAT is an effective quencher for free radicals. The super rice especially Pei'ai 64S/E32 has a higher capacity of total antioxidant and scavenging for active oxygen at high temperature (Fig. 4-A, B). Car is an important antioxidant combined in the membrane, and can eliminate oxygen radicals directly or erase free radicals by destroying the free radical chain reaction. More Car was synthesized in the leaves of super rice than Shanyou 63 under high temperature treatment (Fig. 6).

In conclusion, two super rice Pei'ai 64S/E32 and

Liangyoupeijiu had a better resistance to high temperature than Shanyou 63. The possible mechanisms may be as follows: First, rapid increase in Car, Car/Chl and better antioxidant system were exhibited in the super rice under high temperature stress, which reduced the production and accumulation of active oxygen; Second, the super rice had more effective operation of xanthophylls cycle to dissipate excess excited energy at high temperature; Third, more stable photosynthesis system and higher photosynthetic efficiency were displayed in the super rice than control at high temperature. In addition, the content of heat stable proteins is one key factor for plant to adapt to high temperature<sup>[23]</sup>. The tolerance to high temperature of super rice was improved by increasing heat stable protein content (Fig. 5-B). The mechanism of resistance to high temperature in super rice still remains to be further studied.

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