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Responses of Photosynthetic Functions to Low Temperature in Flag Leaves of Rice Genotypes at the Milky Stage

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Abstract: To examine the chilling resistance of a newly developed super hybrid rice (*Oryza sativa*) Liangyou 122 at the different temperatures, an experiment was conducted to investigate the photosynthetic pigments contents, changes in fatty acids content of thylakoid membrane and the activities of several anti-oxidative enzymes at milky stage with traditional hybrid rice Shanyou 63 as control, by growing rice under the 25/15 ℃ and 25/20 ℃ day/night temperature. The results showed that the malondialdehyde(MDA) content and superoxide anion($O_2^{\bar{\cdot}}$) were increased remarkably, while the activities of superoxide dismutase(SOD) and catalase(CAT) were obviously decreased with the duration of low temperature treatment. Moreover, the change enhanced with the increased difference between day and night temperatures. Meanwhile, the index of unsaturated fatty acid (IUFA) of both varieties also increased. As a result, the photosynthetic rate and the chlorophyll content were decreased considerably, while at beginning the carotenoids content increased and then decreased. Of all the parameters investigated, the variation range in Liangyou 122 was less than that in Shanyou 63, but the values of the former were more than the latter, which means that Liangyou 122 may be more resistant to chilling temperature at the milky stage.

Key words: anti-oxidation system; fatty acid; low temperature; photosynthetic pigments; super hybrid rice

The low temperature is one of the major constrains in rice (*Oryza sativa* L.) production that reduces the grain yield all over the world. In different geographical regions rice varieties often get chilling injury of various degrees and types, especially in the south China, which is double-season rice area in China [1]. Thus, it is crucial to control the abnormal seeding resulting from low temperature and heavy rain [2]. The average temperature of the earth is continuously rising in the past 100 years, however, in the Mid-lower Reaches of the Changjiang River, in some years, rainy and intertropical convergence zone often brings about cold summer [3]. There have been 10 years with a cold summer in the Nanjing region during the last 50 years, and the occurring frequency of cold summer increases yearly, and especially from the late 1980s to the early 1990s (almost once every other year [4]), which could make the summer crops adapting to high temperature easily suffer from precipitate chilling, especially for rice plants sensitive to temperature. Therefore, studying the chilling mechanism of rice has great practical value for stablizing and enhancing rice yield.

Presently, it is widely considered that cell membrane system is the first and the most sensitive part of plants to chilling, so the change in membrane characteristic and the components of membrane are indexes for evaluating plant's cold resistance [5]. The most sensitive period of rice to low temperature is at the seedling and flowering stages. Most of the previous work regarding cold resistance has been done at the seedling stage but few at the flowering stage. Therefore, photosynthetic rate, photosynthetic pigments, fatty acid components of thylakoid membrane and some enzymatic activities of the anti-oxidation system were investigated in a newly-developed super hybrid rice combination Liangyou 122 in order to elucidate the chilling resistance of the super hybrid rice at the milky stage, which may help to breed new rice varieties with greater resistance against chilling.

Liangyou 122 is a two-line hybrid rice

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developed by Jiangsu Academy of Agricultural Sciences in 1998, characterized by strong stem, resistance to lodging, good tolerance to stress, and high yielding [6]. Previously, very little work has been done on cold resistance characteristics of this combination. This experiment is designed to investigate the effects of low temperature on photosynthetic rate, photosynthetic pigments, fatty acid components of thylakoid membrane and some enzymatic activities of anti-oxidation system in Liangyou 122, using a traditional hybrid rice combination Shanyou 63 as control. Shanyou 63 is an popularized rice variety, with low light compensation point, high light saturation point, high net photosynthetic rate of flag leaves, extensive adaptability and so on, and its grain yield basically represents the present rice yield level in China [7-8].

MATERIALS AND METHODS

Materials and experimental treatment

The experimental materials Liangyou 122 and Shanyou 63 were both grown in the Institute of Food Crops, Jiangsu Academy of Agricultural Sciences, Nanjing. At the milky stage, rice plants were uprooted with enough soil in order to decrease the damage to roots and then kept in cultivating containers. During experiment the plants were treated at 25/15℃ and 25/20℃ (average temperature of day and night). The plants were illuminated for 12 h during daytime with a PPFD 1000 μmol/(m^2 • s), and relative humidity 75±5%. After two and four days of treatments, the flag leaves were collected, and the flag leaves corresponding to the four days of treatment from paddy field were as the check. Three replications were used for each treatment.

Photosynthetic rate and respiration rate

Photosynthetic rate and respiration rate were determined with a CIRAS-2 portable photosynthesis measurement system.

Chlorophyll content

The content of chlorophyll was determined by the method of Arnon[9] and Lichtenthaler[10].

Lipid peroxidation

Lipid peroxidation was determined by measuring the malondialdehyde (MDA) content, according to the method of Zhao et al[11].

$O_2^{\bar{\cdot}}$ content measurements

The content of $O_2^{\bar{\cdot}}$ was measured by the method of Wang et al [12]. The absorbance was recorded at 530 nm and used to denote the $O_2^{\bar{\cdot}}$ content according to Elstner [13].

Enzymes assay

SOD activity and CAT activity were measured by the use of reagent box.

Analysis of fatty acids

Lipids were extracted from rice thylakoid membranes with a mixture of chloroform and methanol (1:2; *V*/*V*). The fatty acids were analyzed by the procedure described by Diao et al [14]. The fatty acid analysis was performed on a Hewlett-Packard 1890 gas chromatograph equipped with a hydrogen flame-ionization detector using a 33 m capillary column. The fatty acids were identified based on their retention times compared to known standards.

RESULTS

Changes in photosynthetic rate and respiration rate

It is obvious from Fig.1 that the photosynthetic rate decreased under low temperature conditions. Moreover, the changes in 25/20℃ treatments were lower than that in 25/15℃ treatments, and the 25/15℃ (4 d) treatment showed the most apparent decrease. In comparison with the control plant, the decrease in photosynthetic rate of Shanyou 63 was 80.48%, while that of Liangyou 122 was 79.55% (P=0.0018 for Shanyou 63, P=0.0028 for Liangyou 122). The photosynthetic rates of both combinations decreased markedly with the duration of exposure to low temperature and the difference between day and night temperatures.

As shown in Fig.1, respiration rates had a campanulate changes trend under low temperature.

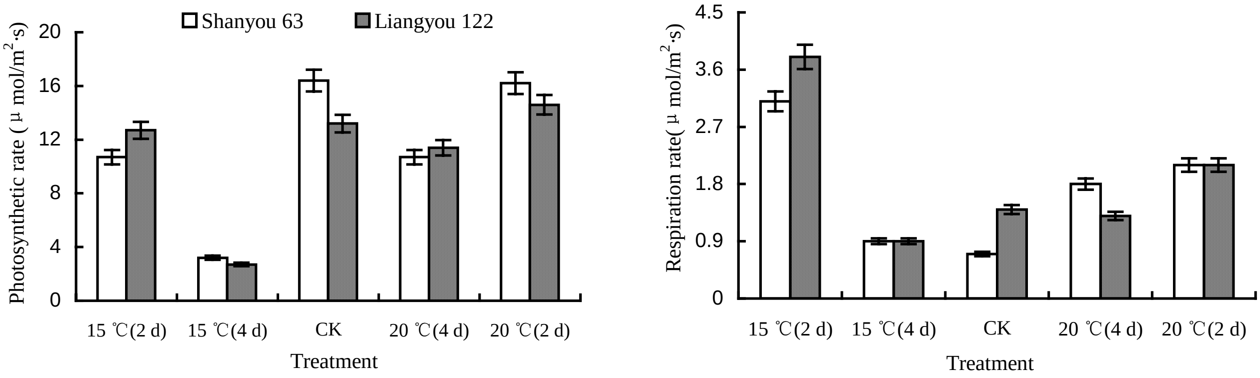

Fig. 1. Changes of photosynthetic rate and respiration rate in flag leaf of rice.

The 25/15℃(2 d) treatment showed the largest initial increase in respiration rate compared with other temperature treatment (*P*=0.0274 for Shanyou 63, *P*=0.0247 for Liangyou 122), and after four days of treatment, respiration rates in Shanyou 63 and Liangyou 122 decreased 70.97% and 76.32% as compared with the two days of treatments, respectively.

Changes in photosynthetic pigments

The changes in photosynthetic pigments are given in Table 1. The data showed that the higher chlorophyll and lower carotenoids contents were observed in Liangyou 122 than in Shanyou 63 (*P*=0.0024). After four days of low temperature treatments, there was a significant reduction in chlorophylls content of both combinations, compared with control plants (Under 25/15℃ treatments, *P*=0.0069 for Shanyou 63, *P*=0.0052 for Liangyou 122), however the effect was not significant after two days of treatment (Under 25/20℃ treatments, *P*=0.9921 for Shanyou 63, *P*=0.3925 for Liangyou 122). Moreover, the carotenoids contents increased at early stage but slightly decreased at later stage. After 2 days of low temperature treatment the decrease has been noted in Chl a/b, but the value increased after four daysof treatment compared with the two days of treatment.

Changes in MDA and O_2^{-} contents

A similar trend was observed in the changes of MDA and O_2^{-} contents under low temperature treatment (Figs. 2, 3). The MDA and O_2^{-} contents of

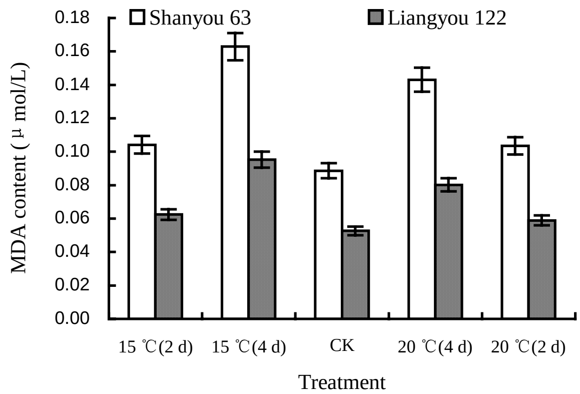

Fig. 2. MDA content in rice flag leaf.

Table 1. Changes in contents of photosynthetic pigments.

Variety	Treatment	Chlorophyll (a+b) (μg/g FW)	Chl a/ b	Carotenoids (μg/g FW)
Liangyou 122	25/15℃ 2 d	718.80±21.45	1.29	21.50±1.39
	25/15℃ 4 d	584.43±85.51	1.39	17.09±7.71
	CK	873.95±44.42	1.47	10.71±3.45
	25/20℃ 4 d	605.80±85.51	1.36	18.50±3.44
	25/20℃ 2 d	842.68±139.48	1.19	21.22±1.46
Shanyou 63	25/15℃ 2 d	692.50±56.58	1.07	46.03±8.73
	25/15℃ 4 d	498.84±93.92	1.27	24.69±11.45
	CK	618.11±92.41	1.34	16.74±3.24
	25/20℃ 4 d	389.94±25.09	1.07	24.86±2.58
	25/20℃ 2 d	618.18±102.42	1.15	40.46±5.63

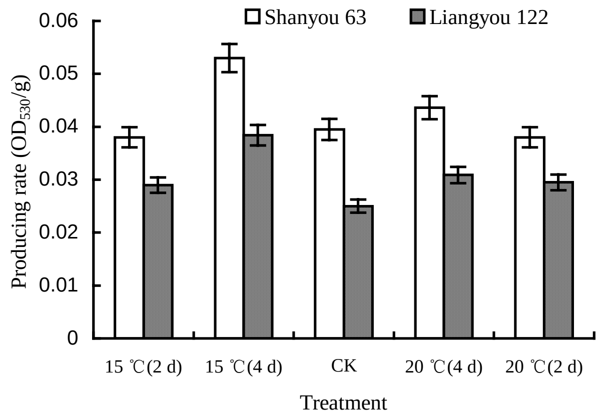

Fig. 3. Changes of superoxide anion in rice flag leaf.

Shanyou 63 were higher than those in Liangyou 122 under low temperature. The MDA and $O_2^{-\cdot}$ contents of both varieties significantly increased with the duration of low temperature stress and the difference between day and night temperatures. After 4-day treatment at 25/15℃ the increase MDA content was 83.72% in Shanyou 63 and 80.87% in Liangyou 122, compared with control plants, while in 25/20 ℃ treatments, the increase was 58.50% and 52.37%, respectively. In contrast the change in $O_2^{-\cdot}$ producing rate was not significant (Under 25/15℃ treatment for four days, P=0.1112 for Shanyou 63，P=0.2123 for Liangyou 122), and the effect of two days of low temperature treatments on $O_2^{-\cdot}$ were more less.

The SOD and CAT activities decreased with the duration of low temperature treatment and increased with the difference between day and night temperatures, but the decrease in Shanyou 63 was greater than that in Liangyou 122 (Fig. 4). After treatment at 25/15℃ and 25/20℃ for four days, the SOD activity in Shanyou 63 decreased by 53.68% (P=0.0084) and 50.65% (P=0.0093), respectively, in comparison with control plant, while the decrease in CAT activity was 52.24% (P=0.0092) and 40.98% (P=0.0117), respectively. On the other hand, the decline in SOD activity of Liangyou 122 was 19.19% (P=0.1590) and 13.30% (P=0.2371), while the CAT activity declined by 32.10% (P=0.0545) and 24.01% (P=0.0778), respectively. Moreover, the effect of 2-day treatment on Shanyou 63 was larger than that on Liangyou 122.

Changes in composition of fatty acids in thylakoid membrane

The different rice combinations have similar fatty acid components, and mostly are myristic acid(14:0), palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3). Of all the components, palmitic acid, oleic acid and linoleic acid contents were higher than the others, while myristic acid content was almost negligible. This is in agreement with the results of Chen et al[15]. It is clear from Table 2 that the index of unsaturated fatty acid (IUFA) of thylakoid membrane ascended with the duration of low temperature and the increased difference between day and night temperature. After treatments at 25/15℃ and 25/20℃ for four days, the increase in IUFA of Liangyou 122 was 34.04% and 25.71%, respectively, compared with control plants, while the increase in

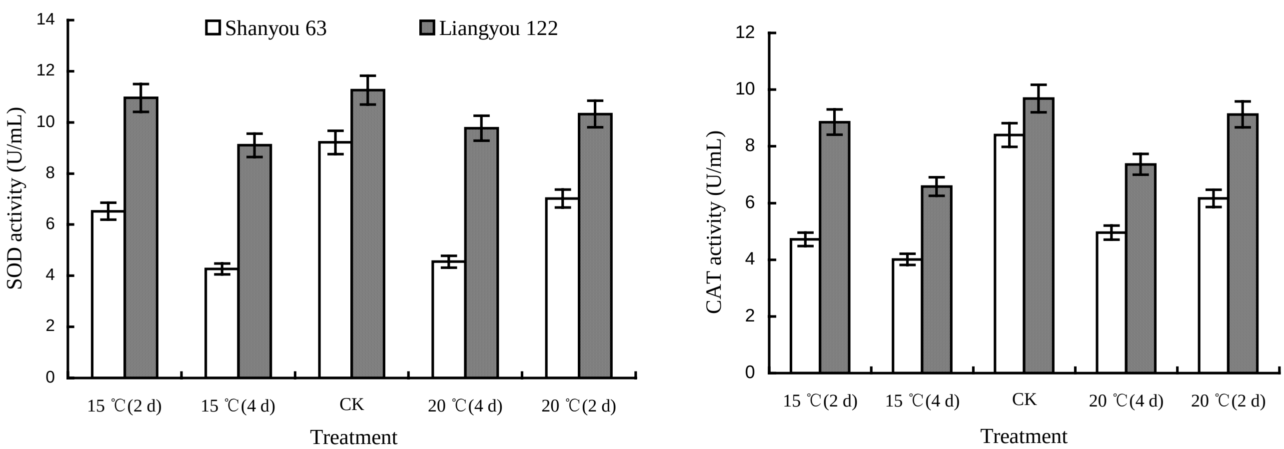

Fig. 4. Changes of SOD and CAT activities in flag leaf of rice.

Table 2. Composition of fatty acids in rice thylakoid membrane.

Combination	Treatment	Fatty acid (mol/%)					
		16:0	18:0	18:1	18:2	18:3	IUFA
Liangyou 122	25/15℃ 2 d	29.48	4.37	5.34	14.94	45.87	172.83
	25/15℃ 4 d	27.67	3.79	4.13	17.93	46.48	179.43
	CK	38.87	7.78	6.07	14.05	33.23	133.86
	25/20℃ 4 d	31.91	5.15	3.14	14.26	45.54	168.28
	25/20℃ 2 d	31.51	6.52	3.71	14.56	43.70	163.93
Shanyou 63	25/15℃ 2 d	41.43	13.35	12.86	8.25	24.11	101.69
	25/15℃ 4 d	32.75	10.44	7.49	14.82	34.50	140.63
	CK	54.53	11.36	10.68	8.02	15.41	72.95
	25/20℃ 4 d	46.45	8.91	8.08	12.80	23.76	104.96
	25/20℃ 2 d	54.78	13.93	10.16	8.59	13.72	68.51

IUFA (index of unsaturated fatty acid) = [(18:1)+(18:2)×2+(18:3)×3]×100[15].

Shanyou 63 was 92.78% and 43.88%, respectively.

DISCUSSION

Low temperature stress often causes decrease in photosynthetic rate and increase in respiration rate at early stage of plant growth [5]. Similar results were also observed in the present study (Fig.1). It has been observed that after treatment at 25/15℃ for four days, the photosynthetic rates of Shanyou 63 and Liangyou 122 dropped significantly with an increase in respiration rate, but the decrease has been noted after treatment for two days. There was no significant effects on photosynthetic rate and respiration rate under 25/20℃ treatment, this may be attributed to no obvious difference in temperature between the treatment (25/20℃) and control. In Shanyou 63 the decrease in photosynthetic rate due to low temperature was larger than that in Liangyou 122, and vise verse in respiration rate. The differences in photosynthetic and respiration rates indicate that Liangyou 122 could have higher CO_2 utilization efficiency than Shanyou 63 under low temperature conditions.

The cell membrane is mostly responsible for adaptation of plants to the changes in temperature, especially for the cytoplasm membrane and thylakoid membrane. Chloroplast thylakoid membrane is a basic photosynthetic membrane system with two membrane layers, which contains light harvesting complexes and light reaction centers [16]. The IFUA and fluidity of membrane is closely related to plant cold-resistance. The enhancement of unsaturated fatty acids may increase the fluidity of the cell membranes during exposure to low temperature stress and the lower temperature of the phase transition, thus resulting in an increase of resistance against chilling [5]. The results of the current work (Table 2) also supported this point that low temperature caused the increase in unsaturated fatty acids mainly due to linolenic acid and the decrease in saturated fatty acids due to palmic acid. After four days of treatment at 25/15℃, the increase in unsaturated fatty acids of Liangyou 122 and Shanyou 63 were 28.47% and 66.55%, while the decrease in saturated fatty acids were 32.56% and 34.45%, respectively, in comparison with control plant. The changes in unsaturated and saturated fatty acids of Shanyou 63 were larger than those in Liangyou 122 with smaller IFUA than that of Liangyou 122. Therefore, it can be suggested that the higher unsaturated fatty acids in Liangyou 122 may be responsible for resistance to chilling. However, the fatty acids of thylakoid membrane in Shanyou 63 changed at greater extend to adapt low temperature, indicating that Shanyou 63 was more sensitive to chilling stress at the filling stage, whereas Liangyou 122 more resistant to low temperature. Similar results were reported by Ji et al [17] and Yang et al [18].

Plant would produce much reactive oxygen species (ROS) and hydroxyl radicals under low temperature [19]. The increase in ROS and hydroxyl radicals causes lipid peroxidation, which brings remarkable enhancement of MDA content [20]. Under normal conditions, ROS scavenger system of plants such as SOD, CAT and POD can scavenge ROS, and protect photosynthesis apparatus, membrane and biology function molecules [21]. It can be seen from Fig. 5 and Fig. 6 that SOD and CAT activities were not obviously changed after two days of low temperature treatment, indicating that protection enzyme system was still in function. After four days of treatment, the SOD and CAT activities dropped notably, while the MDA content and $O_2^{-\cdot}$ increased obviously, which indicated that the producing and scavenging of ROS were in unbalance state, causing significant decreases in photosynthetic rates and respiration rates, and consequently affected the growth of rice, even led to the death of rice plants. The formation of ROS and hydroxyl radicals directly or indirectly affects the chlorophyll content, especially chl a, thus resulting in the decrease in ratio of Chl a to b [22]. Similar results were also observed in our study and by Zeng et al [1]. In rice, Chl a mainly accumulates in PSII reaction center and Chl b in light harvesting complex (LHC) [23], and the ratio of Chl a and Chl b can be used to present the state of PSII reaction. Chl a/b in Liangyou 122 was always higher than that in Shanyou 63, indicating that PSII reaction center in Liangyou 122 was more steady under low temperature at the filling stage. Moreover, with the duration of low temperature and the increased difference between day and night in temperature, chlorophyll content decreased gradually and finally leaf photosynthesis declined [24]. We have noted that the carotenoids content increased at beginning and then dropped slightly. The carotenoids can participate in intercepting excess light energy absorbed by photosynthetic pigments in plant photosynthetic organs, thus enhancing the cold resistance [1]. Therefore, it could be assumed that the changes in carotenoids content may play a major role in an adaptation of rice plant to low temperature stress.

In summary, due to low temperature treatment, the unsaturated fatty acids of the two rice combinations increased, together with lipid peroxidation and free-radicals contents, but photosynthesis and protection enzymes activities decreased remarkably. Of all the characteristics investigated, the changing in quantitative characteristics of Liangyou 122 less than that in Shanyou 63, but the quantitative characteristics of the former was more than the latter, providing a physiological base that Liangyou 122 is more resistant to chilling temperature at the flowering stage [5, 25]. It can be further suggested that the new super hybrid rice Liangyou 122 has broader range of cultivation such as high latitude or high altitude, and Liangyou 122 with strong cold resistance has a more steady yield than Shanyou 63 in various climate conditions. The world climate will get warmer in the future, and the damage caused by low temperature would be much more severe. The mechanism of chilling injury to plant is still not very clear; correspondingly the adaptability of plant to chilling is various. Changes in composition of fatty acids, membrane protection system and photosynthetic function are pivotal aspects, so researching in these facets has great significance. We can expect even greater cold resistant rice varieties in response to these factors. It can be concluded from our experiments that short-term low temperature may not influence the grain yield of Liangyou 122 at flowering, but it is necessary to take more precautions to ensure the higher yield under long-term chilling conditions.

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