

Effects of Chilling Stress on Photosynthetic Rate and Chlorophyll Fluorescence Parameter in Seedlings of Two Rice Cultivars Differing in Cold Tolerance

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Abstract: A cold-tolerant cultivar, Xiangnuo 1, and a cold-sensitive cultivar, IR50, were used to study the influence of chilling on photosynthetic rate and chlorophyll fluorescence parameters in rice seedlings. The photosynthetic rates declined dramatically during chilling, and decreased by 48.7% and 67.5% in Xiangnuo 1 and IR50 seedlings, respectively, after being subjected to chilling treatment for two days. Chlorophyll fluorescence measurements showed that relatively higher q_P and q_{NP} in Xiangnuo 1 were maintained to dissipate the redundant excitation energy and protect the reaction centers from chill injury; accordingly, redundant excitation energy accumulated less in the reaction centers, and antenna systems were less injured by chilling in Xiangnuo 1. On the contrary, in IR50, q_P and q_{NP} declined rapidly while E_x increased, as the chilling persisted. This result indicated that the reaction centers and antenna systems in IR50 were damaged severely by chilling, which led to the lower photosynthetic rate.
Key words: chilling; rice; photosynthetic rate; chlorophyll fluorescence analysis

It has been reported that rice showed damage from chilling when the temperature was lower than 10–12℃ [1]. The injury to metabolism and physiological process in rice upon chilling is irreversible, including the increased membrane permeability, inhibition of chlorophyll synthesis [2], the broken chloroplast [3], and eventually the decreased photosynthetic ability. Moreover, it was investigated that the photo-inhibition more easily occurred under low temperatures both in cold-tolerant and cold-sensitive plants [4]. Chilling lowered the activity of dark reaction in chloroplast stroma [5], reduced the light-dependent reaction activity in thylakoid membrane [6], decreased the light energy-transferring and converting efficiency of PSⅡ in the thylakoid membrane [7], and reduced CO_2 assimilation activity. Furthermore, the activity of the light-dependent reaction was more sensitive to chilling than that of the dark reaction [6]. Kaniuga [8] reported that chilling reduced the activity of Hill reaction, and inhibited the electron transmission activity. Moreover, the inhibition happened mainly at the oxidation side of PSⅡ [9–11]. As we known, excessive excitation energy increased in plant under chilling stress. This energy could be dissipated through various paths such as the dissipation and the heat emission (NPQ) in photochemical reaction of photosynthesis. Also, a relevant study showed that under medium illumination at 4℃, excessive light energy could be dissipated through photochemical mechanism in barley. Consequently, excessive light energy could be dissipated via the xanthophyll cycle in barley and in the cold-tolerant rice cultivars, but not in the cold-sensitive rice cultivars. This discrepancy suggested the mechanism responsible for the cold-sensitive phenomena occurrence [12]. There are still no reports about the difference of light energy allocation and excessive excitation energy dissipation between these two rice cultivars upon chilling. In present study, photosynthetic rate and chlorophyll fluorescence were measured in rice cultivars with different cold-tolerance. We expected to speculate the relationship between difference on photosynthetic ability of rice cultivars and the differences on energy allocation and excessive excitation energy dissipation upon chilling.

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MATERIALS AND METHODS

Plant materials

Rice (*Oryza sativa* L.) cultivars Xiangnuo 1 (cold-tolerant) and IR50 (cold-sensitive) were cultured to three-leaf stage with the method described by Liu et al [13] and then were transferred to an air-conditioned chamber (8℃) with a photon flux density (PFD) of 160 μmol/(m^2 •s) and 80% relative humidity. The control was set under normal temperature (28℃) in another chamber with the same PDF and relative humidity. After chilling treatment for five days, the treatment was transferred to the control conditions for two additional days under control conditions. The rice leaf for determination was randomly sampled every day after exposed to chilling.

Photosynthetic rate

TPS-1 portable photosynthetic measurement system (PP-Systems, UK) was used to measure the photosynthetic rate. When measuring, the light intensity above leaf room was 800–1000 μmol/(m^2• s), air current velocity was 300 mL / min in the leaf room, and the photosynthetic rate was expressed by P_n [μmol CO_2 / (m^2 • s)].

Chlorophyll fluorescence analysis

A pulse-modulated fluorometer FMS-1 (Hansatech, UK) was used to analyze the dynamic fluorescence parameters. Initial fluorescence yield F_o was measured under the examining light [<0.05 μmol /(m^2 • s)] after the leaves were set in the dark to adapt for 20 min. Then F_v/F_m was measured under the saturated pulse light [12 000 μmol /(m^2 • s)]. The inside source turning light [180 μmol /(m^2 • s)] was turned on, examining light and saturated pulse light (1 pulse) were used alternatively to measure maximum fluorescence F_m'. Then the function light was closed and far infrared was used immediately to measure F_v'/F_m' and F_o' in light when the ΦPSⅡ kept stable through recorded fluorescence parameters and fluorescence quenching curve periodically. All parameters were measured repeatedly 5 times. According to the methods of Schreiber [14] and Adams [15], the parameters were calculated based on the formula below:

$$L_{(PFD)}=1-(q_P \cdot F_v'/F_m')/0.83,$$

$$P=F_v'/F_m' \cdot q_P,$$

$$D=1-F_v'/F_m',$$

$$E_x=F_v'/F_m' \cdot(1-q_P).$$

RESULTS

Changes of photosynthetic rate

P_n decreased by 48.7% and 67.5% in Xiangnuo 1 and IR50 after they were subject to chilling for two days, respectively. P_n decreased by 85.0% and 94.4% in the two cultivars after five days, respectively. When they recovered under control conditions for two days, P_n in Xiangnuo 1 increased to the control level, while P_n in IR50 continued to decline until only weak respiratory rate could be detected (Fig. 1).

Analysis of chlorophyll fluorescence

Generally, F_v/F_m is used to reflect the original photochemical efficiency of PSⅡ. F_v/F_m decreased in the two rice cultivars, but severely in IR50. When two cultivars were subject to chilling for three days or five days, F_v/F_m in Xiangnuo 1 decreased by 26.2% and 40.3%, whereas, in IR50 it decreased by 46.6% and 69.2%. After they recovered under control conditions for five days, F_v/F_m in Xiangnuo 1 returned to original level, but it did so more slowly in IR50 (Fig. 1).

q_P is a ratio of opened reaction centers to total reaction centers in PSⅡ. Upon chilling, q_P in Xiangnuo 1 declined rather slowly, whereas in IR50 it declined continuously by 25.8% and 51.1% after chilled for three and five days, respectively. Eventually, q_P could recovered to the control level in both cultivars when they were returned to control conditions (Fig. 1).

q_{NP} in Xiangnuo 1 and IR50 was greater than the control upon chilling. Chilling-induced changes in q_{NP} was little in Xiangnuo after chilled for one day, but it increased gradually and above the control level from an onward. In contrast, q_{NP} in IR50 increased rapidly after chilling for one day. Then it decreased gradually as the chilling continued. Eventually, q_{NP} could recover in both cultivars when they were under control conditions (Fig. 1).

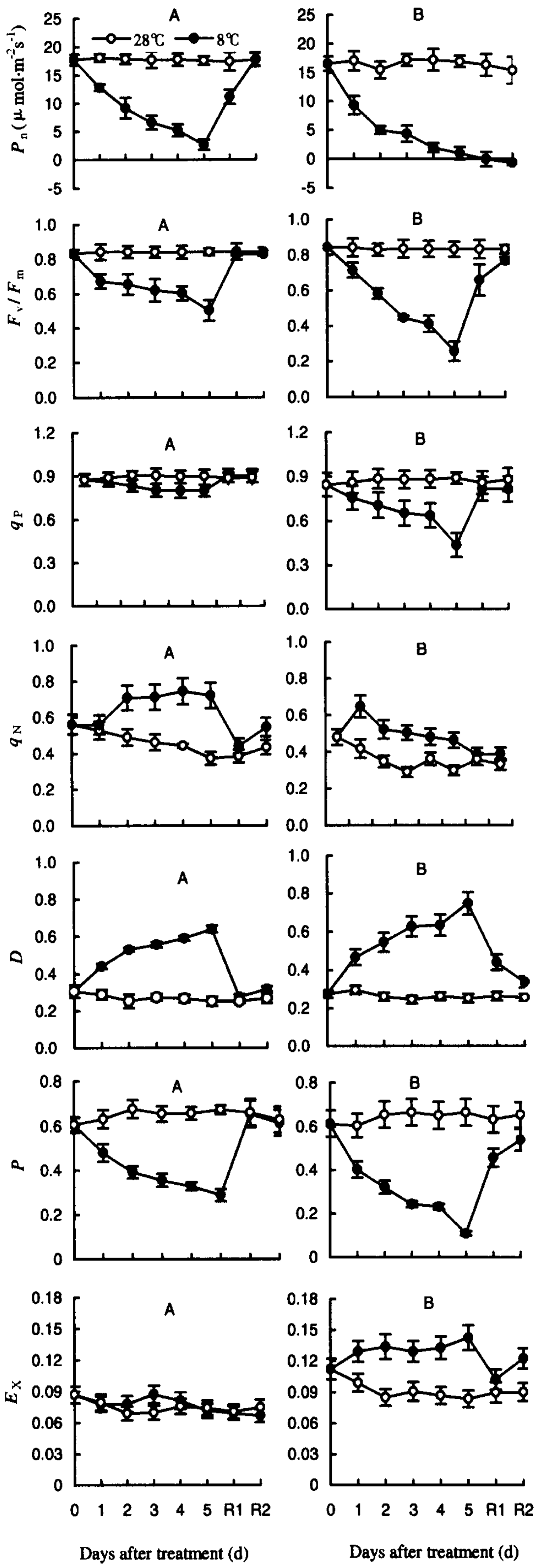

Fig. 1. Photosynthetic rate and fluorescence parameters of rice cultivars Xiangnuo 1(A) and IR50(B) under chilling treatment and after restoring at room temperature.

R1, R2 refer to 1 d and 2 d after restoring at room temperature, respectively.

The light energy absorbed by the plant leaf can be divided into three parts according to the fluorescence parameter. The first is that energy dissipated through the antenna system called *D*. The second is used in the photochemical reaction called *P*; the last is depleted through the photochemical reaction in reaction centers called E_x. In this study, *D* increased in both cultivars as the chilling continued, *D* increased non-significantly by 60.6% and 66.4% in Xiangnuo 1 and IR50, respectively. Consequently, *D* recovered in Xiangnuo 1 under control conditions for one day, but it needed two days in IR50 (Fig. 1).

The value of *P* decreased rapidly by 45.8% and 63.4% in Xiangnuo 1 and IR50 upon chilling for three days, and by 57.2% and 83.5% after five days, respectively. In case of return to control conditions, *P* recovered to the control level faster in Xiangnuo 1 than in IR50 (Fig. 1). In contrast with *D* and *P*, E_x was lower in both cultivars. E_x only significantly increased in IR50 upon chilling (Fig. 1).

DISCUSSION

Of all the physiological processes, photosynthesis is affected heavily by chilling. When the rice seedlings were chilled, the superstructure of chloroplasts is damaged severely [3], and the electron transfer function decreased [10]. It was reported that in Xiangnuo 1, membrane permeability kept stable at three-leaf stage at 8℃ for five days, whereas, it increased continuously in IR50, which meant that Xiangnuo 1 was more tolerant to chilling than IR50[16]. In this research, photosynthetic rate decreased in both cultivars, and it was higher in the cold-tolerant cultivar Xiangnuo 1. As we know, F_v/F_m values are used to estimate the original photochemical efficiency in PS II [17], which directly influence the photosynthetic rate under low light intensity[18]. A study by Li[19] on chlorophyll fluorescence in flag leaves in indica rice varieties with different cold-tolerance suggested that F_v/F_m decreased more rapidly upon chilling in the light than in the dark. Moreover, F_v/F_m decreased more in the cold-tolerant cultivar than in cold-sensitive cultivar, but it recovered quickly under normal temperature with weak light. In this study, F_v/F_m decreased in both cultivars upon chilling with weak light, but it decreased more severely in the cold-sensitive cultivars, indicating that the decrease of F_v/F_m was consistent with the tolerance to chilling in rice cultivars, which was also consistent with the study on tomato cultivars with different cold-tolerance [20].

The utilization of light energy in plant includes energy transfer through the photochemical reaction, the production of non-photochemical heat, and excessive energy dissipated in the form of chlorophyll fluorescence. A change in the photochemical reaction and heat dissipation could cause change of chlorophyll fluorescence quenching, which consists of photochemical and non-photochemical quenching. It was reported that the activity of reaction centers and electron transfer antennae in PSⅡwere affected by chilling, which would lead to photoinhibition in PSⅡ because of photo-damage to reaction centers and the decrease of ability in trapping excitation energy in reaction centers [21]. The study showed that non-photochemical quenching was a self- protective mechanism that played a role in protecting the photosynthetic apparatus and that was helpful to resist photoinhibition upon chilling. Thus, it was regarded that photoinhibition was caused by inefficient NPQ path in photoinhibition-sensitive rice [12]. In our study, the energy allocated to photochemical reaction decreased. While the energy allocated to dissipation in antennae and reaction centers increased. The result indicated that during the decrease of photosynthetic rate in rice, excessive dissipation energy increased in photosynthetic apparatus. Therefore, the heat dissipation increased in the antenna system, with less decrease of energy in the photochemical reaction. On the other hand, the injury to light reaction centers was less in the cold-tolerant rice cultivar, which led to higher photochemical efficiency and less energy dissipation allocated to antenna system. At the same time, this study indicates higher photochemical efficiency as well as increasing q_{NP} in Xiangnuo 1 both help to dissipate the redundant energy, which may be the reason that it was more tolerant to chilling and had higher photosynthetic efficiency. Whereas, in IR50 q_P decreased more and the q_{NP} route did not developed efficiently. This probably was because partial reaction centers were inactive and the antenna system was damaged upon chilling. Therefore, the photosynthetic ability was suppressed in IR50 by aggregation of excessive excitation energy.

There were multiple effects on physiological and biochemical mechanisms and photosynthesis in rice seedlings upon chilling. Not only the change of fluorescence spectrum could be affected by the change in leaf color, leaf components, and membrane lipids, but also the photosynthetic rate could be affected by the change in the metabolism of photosynthetic enzymes. Consequently, in this paper, it was just a simple speculation of the change of photosynthetic rate according to the change of PSⅡanalyzed by fluorescence parameters, and further study is needed to interpret their relationship. Moreover, further study is also needed to show whether the chilling played a direct effect on photosynthetic rate and chlorophyll fluorescence parameters.

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