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# Photochemical efficiency of PS_and membrane lipid peroxidation in leaves of *indica* and *japonica* rice (*Oryza sativa* L.) under different temperatures and light intensities

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## Introduction

Exposure of plant leaves to excessive light can result in reduction in quantum yield. This is known as PI. Long-term exposure of plant to strong light with stress such as chilling temperature can result in photo-destruction of photosynthetic pigments. This destruction is defined as PO. The primary site of PI had been suggested to be located in PS_ reaction center. The states and the re-synthesized capacity of D1 protein were the physiological basis in the course of PS_ recovery. Numerous studies showed that there were multiplex protective mechanisms, such as xanthophyll cycle, active oxygen scavenging system, photorespiration, against photodamage in photosynthetic apparatus. In recent years, many studies on PI or PO in plants were carried out and gave varied results, for the photoinhibitory conditions were different. The aims of this contribution are to explore the differences in traits related to PI and PO between *indica* and *japonica* rice, the physiological relationships between PI and PO under different temperatures and PFDs. So as to provide physiological basis for the genetic improvement approach to hybrid rice with PI- or PO-tolerance.

## Materials and methods

PO-tolerant *japonica* rice cv. 9516 and PO-sensitive *indica* rice cv. Shanyou 63 were planted in the pots in a greenhouse under natural sunlight conditions with routine manage. During ear stage potted rice plants were put in phytotron with day/night temperature of 26–1_/ 22–1_ (moderate temperature) and 11–1_/ 10–1_ (chilling temperature), and relative humidity of 78~82%. The photoperiod was 12 h, with the PFDs of 350, 700, 1050 and 1400 _mol $m^{-2}$ $s^{-1}$ , respectively, from 6:00 to 18:00 for 4 days. There were 2 groups (total of 8 treatments), replicated 3 times. Xanthophyll cycle components were assayed by HPLC system. A pulse modulation fluorometer (FMS-2, Hansatech, UK) was used to measure the chlorophyll fluorescence parameters. Measurements of D1 protein contents, SOD activities, $O_2^-$ generation rates and MDA contents were carried out.

## Results

*Effect of different temperatures and PFDs on* $F_v/F_m$*, D1 protein contents, xanthophyll cycle and MDA in* indica *and* japonica *rice.*

Photochemical efficiency of PS_ ($F_v/F_m$) in the leaves of *japonica* rice cv. 9516 and *indica* rice cv. Shanyou 63 decreased to some extent with the increasing PFDs. Under moderate temperature and higher PFDs (1050-1400_mol $m^{-2}$ $s^{-1}$) for 4 days, $F_v/F_m$ decreased slightly (3.38--6.47%) in *japonica* rice but obviously (9.17-23.01%) in *indica* rice. PI of photosynthesis happened in leaves of *indica* rice. Under chilling temperature and higher PFD, the extents of decrease in $F_v/F_m$ were enhanced, indicating

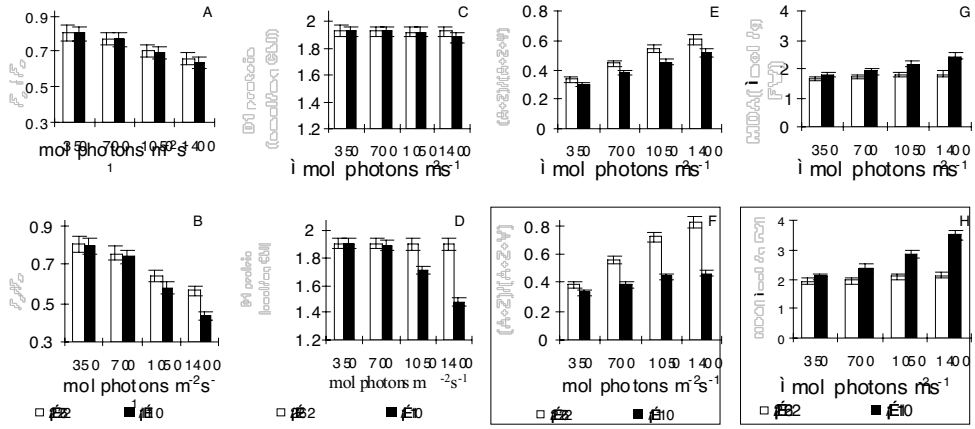


**Fig.1 The changes in PS ¥ ²photochemical efficiency ( $F_v/F_m$), D1 protein contents, (A+Z/(A+Z+V) and MDA in leaves of *japonica* rice cv.02428 (upper) and *indica* rice cv. 3037 (lower) under different temperatures and light intensities for 4 days.**

that grievous PI happened and *indica* rice suffered from more PI than *japonica* rice (Fig. 1A, B). No obvious changes in D1 protein contents in both *indica* and *japonica* rice were observed under moderate temperature and different PFDs. However, under chilling temperature and higher PFDs D1 protein contents decreased by 10.13--21.97% in *indica* rice, but did not change obviously in *japonica* rice (Fig. 1C, D). Xanthophyll cycle pool, total contents of violaxanthin (V), antheraxanthin (A) and zeaxanthin (Z), was unchanged at different temperature and PFDs. However, A and Z contents increased with the increasing PFDs in both rice under moderate temperature. So the (A+Z)/(A+Z+V) ratio increased with increasing PFDs. On the contrary, A and Z contents decreased under chilling temperature, especially with higher PFD illuminated. Therefore, (A+Z)/(A+Z+V) ratio decreased and xanthophyll cycle weakened greatly. Furthermore, effect of temperatures and PFDs on (A+Z)/(A+Z+V) was more obvious in *indica* rice than in *japonica* rice (Fig. 1E, F). No obvious changes in lipid peroxidation product--- malondialdehyde (MDA) in leaves were observed although PI happened such as in *indica* rice under moderate temperature and different PFDs. However, under chilling temperature, MDA contents increased with the enhancing PFDs, for example, MDA contents increased by 20.67-30.07% in *japonica* rice and 37.50-66.35% in *indica* rice in higher PFDs, indicating that PO took place. The difference in MDA contents between two rice cultivars showed that *indica* rice suffered more disastrous PO than *japonica* rice (Fig.1G, H).

*Dynamic changes in* $F_V/F_m$*, D1 protein contents, xanthophyll cycle and MDA in* indica *and* japonica *rice under chilling temperature and higher PFD.*

Under chilling temperature and higher PFD, $F_V/F_m$ in *indica* and *japonica* rice declined constantly with the time delayed. On the 4th day, $F_V/F_m$ decreased by 21.63% in *japonica* rice and by 39.25% in *indica* rice (Fig.2A,B). Non-photochemical quenching (qN) decreased distinctly by 41.40% and 19.1% on the 1st day, and continued to decline on the other days, and decreased by 56.3% and 27.3% on the 4th day in *indica* and *japonica* rice respectively. The MDA contents increased by 29.3% and 12.6% on the 1st day, and by 74.3% and 46.9% on the 4th day in *indica* and *japonica* rice respectively (Fig. 2G, H), while light energy conversion was obstructed and qN decreased. The tendency of change in $O_2^-$ generation rates was consistent with

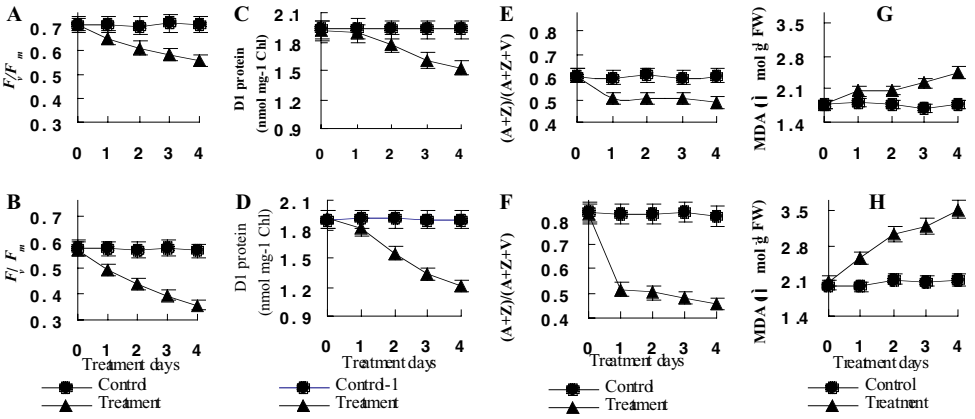


**Fig.2 Dynamic changes in** $F_v/F_m$**, D1 protein contents, (A+Z)/(A+Z+V) and MDA in** ***japonica*** **rice cv. 9516 (upper) and** ***Indica*** **rice cv. Shanyou 63 (lower) under chilling temperature and higher PFD. Control: 26 ¡æ /22 ¡æ 1050 |¦ mol m $^{-2}$ s$^{-1}$; Treatment: 11 ¡æ/10 ¡æ 1400 |¦ mol m $^{-2}$ s$^{-1}$.**

MDA. The difference in MDA contents and $O_2^-$ generation rates between two rice cultivars showed that *indica* rice was more sensitive to PO than *japonica* rice. D1 protein contents declined by 5.0% and 2.3% on the 1st day, and by 41.3% and 22.5% on the 4th day in *indica* and *japonica* rice_ respectively (Fig. 2C, D), which was consistent with the change in $F_V/F_m$. Xanthophyll cycle pool, total contents of V, A and Z, was unchanged during the treatment days. And yet, the (A+Z) contents descended by 36.8% and 15.7% on the 1st day, and kept on decreasing, and by 56.8% and 22.7% on the 4th day in *indica* and *japonica* rice, respectively (Fig.2E, F). In addition, super oxide dismutase (SOD) activities dropped obviously, especially in *indica* rice under chilling temperature and higher PFDs.

*Effects of inhibitors on* $F_v/F_m$ *under different temperatures and PFDs*

The inhibitory effects of Streptomycin (SM, a D1 protein synthesis inhibitor), dithiothreitol (DTT, xanthophyll cycle inhibitor) and diethyldithiocarbamate (DDTC, SOD inhibitor) on $F_v/F_m$ were more serious under chilling temperature than under moderate temperature, and in *indica* rice than in *japonica* rice. Above-mentioned facts showed that there were coordinated changes and/or some causalities among D1 protein, xanthophyll cycle and $F_v/F_m$. It was evident that the synthesis of D1 protein, xanthophyll cycle and SOD activities, which played some roles in raising $F_v/F_m$, were physiological basis of PI- and PO-resistance in rice plants.

## Discussion

Plants under environmental stress often make the injury to themselves minimized by endogenous protective mechanisms such as thermal dissipation of non-radiation and

acceleration of xanthophyll cycle for dissipating excessive excitation energy if under higher PFD. However, under chilling temperature with higher PFD, light energy conversion efficiency of photosynthetic apparatus declines significantly, and more excessive excitation energy needs dissipating. At the same time, the activity of violaxanthin deepoxidation (VD) enzyme was restricted and the VD weakened. Xanthophyll cycle-dependent qN also reduced physiologically. The inhibition of xanthophyll cycle resulted in serious PI. So the PI happened more seriously with increasing in equal PFD under chilling temperature than under medium temperature, namely, chilling temperature made the plants more sensitive to high PFD. In addition, the full reduction of the acceptive site of PS_under chilling temperature was of great benefit to recombination of ion pair $P_{700}^{+}/A_o^{-}$ or $P_{700}^{+}/A_1^{-}$ and forming the triplet $P_{700}$. Furthermore $O_2$ reacted with triplet chlorophyll to form toxic $^1O_2$ which attack SOD directly. It was considered that a great deal of increase in active oxygen and a mass of decrease in activities of scavenging active oxygen system, and further resulted in membrane lipid peroxidation and PO occurred.

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## References

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