BIOLOGIA PLANTARUM 54 (1): 164-168, 2010

BRIEF COMMUNICATION

Characteristics of chlorophyll fluorescence and antioxidative system in super-hybrid rice and its parental cultivars under chilling stress

Y.H. ZHANG[1,2], L.J. CHEN[1], J.L. HE[1], L.S. QIAN[1], L.Q. WU[1] and R.F. WANG[1]*

Anhui Agriculture University, Hefei 230036, P.R China[1]
School of Life Sciences, Nanjing University, Nanjing, 210093 P.R. China[2]

Abstract

Characteristics of chlorophyll fluorescence and antioxidative system were investigated in rice (*Oryza sativa* L.) super-hybrid Liangyoupeijiu (LYPJ), maternal cultivar Peiai64s, and paternal cultivar indica rice 9311 under chilling stress. During 6-d chilling treatment, chlorophyll content of all three genotypes was gradually declined. However, the decrease in photosystem 2 (PS 2) maximum photochemical efficiency (F_v/F_m) and quantum yield of PS 2 (Φ_{PS2}) was less expressive in LYPJ than in parental cultivars The electrolyte leakage and malondialdehyde content in all cultivars increased after chilling treatment, but LYPJ exhibited the least increasing tendency. Activities of superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR) were higher in LYPJ than in parental cultivars. The results demonstrated that tolerance to chilling stress in LYPJ might be adopted mostly from its maternal cultivar.

Additional key words: APX, chlorophyll content, electrolyte leakage, F_v/F_m, GR, photosystem 2, SOD.

Rice is an important agricultural crop and its growth in the early and late development stage suffers from chilling damage in combination with high sun irradiance. One major factor of chilling sensitivity in higher plants is the impairment of photosynthesis and photosystem 2 (PS 2) is particularly vulnerable to damage (Krause and Weis 1991). This is partly because lower temperature generally reduces the rates of carbon dioxide reduction and photorespiration and therefore limits the sinks for the absorbed excitation energy (Allen and Ort 2001). In addition, the excess excitation energy, which is not promptly quenched, accelerated the formation of reactive oxygen species (ROS), resulting in the reduction of the photosynthetic efficiency (Foyer *et al.* 1994). In order to avoid severe photooxidative damage, higher plants developed defense system to scavenge ROS, consisting of several enzymes, such as superoxide dismutase (SOD), ascorbate peroxide (APX), and glutathione reductase (GR) (Bowler *et al*. 1992). Higher contents of defense enzymes were found to be correlated with higher chilling tolerance of cucumber radicles (Kang and Saltveit 2002). While photosynthetic characteristics and the role of ROS scavenging enzymes under abiotic stress in many other crops have been well documented (Bertamini *et al.* 2006, Kočová *et al*. 2009), very few data are yet available on relationships of hybrid rice and its parental cultivars. In the present study, the chill tolerance of super-rice hybrid, LYPJ, and its parental cultivars were characterized. Furthermore, stress-induced changes in photosynthetic characteristics and the possible roles of anti-oxidative system under chilling stress were also assessed.

Seeds of the rice (*Oryza sativa* L.) super-hybrid LYPJ (Peiai64s × 9311) and its parents (PA64s and 9311) were presoaked in a water bath at 30 °C overnight and then covered with fully wet gauze cloth for germinating at 25 °C. The germinated seeds were transplanted in 30 cm high pots with a diameter of 25 cm when the plants were about 15 cm high. All plants were then grown outdoors under natural irradiance (13 - 14-h photoperiod, day/night temperature of 30 - 36/26 - 33 °C with a sufficient supply

Received 31 May 2008, *accepted* 27 October 2008.
Abbreviations: APX - ascorbate peroxidase; F_v/F_m - variable to maximum fluorescence ratio (PS 2 maximum photochemical efficiency); GR - glutathione reductase; MDA - malondialdehyde; NPQ - non photochemical quenching; ROS - reactive oxygen species; SOD - superoxide dismutase; Φ_{PS2} - the actual PS 2 efficiency.
Acknowledgements: We thank Mr. Zhulong Chan for linguistic revision of the manuscript. Illuminating comments from the editors and anonymous reviewers are also appreciated. Research was supported by the Major and Key fund of Anhui province education department and High school youth fund of Anhui province education department (2008jq1052 and 2008jq1045zd). The first two authors contributed equally to the paper.
* Corresponding author; fax: (+86) 551 5786935; e-mail: rongfuwang@hotmail.com

of water and fertilizer. At the heading stage, one group of plants was subjected to chilling treatment of 15 °C for 6-d in phytotron under 12-h photoperiod with irradiance of 400 μmol $m^{-2}s^{-1}$ and 70 % relative humidity, while the other group of plants was grown outdoors. Samples of the leaves were taken on 2nd, 4th and 6th day after chilling treatment and the flag leaf was used for the following analyses. All parameters were measured with at least five repetitions, and the whole experiments were repeated twice.

Ten leaf discs were cut from the flag leaves and extracted in 5 cm^3 of 80 % acetone solution in the dark at 25 °C till fully blanched. The concentrations of the total chlorophyll were determined according to Arnon (1949), where absorbance was measured at wavelengths 663 and 645 nm using spectrometer (*756MC UV*, *Shanghai Analysis Instrument Co*., Shanghai, China). A pulse-modulated fluorometer (*FMS-2*, *Hansatech Instruments*, Norfolk, UK) was used to measure chlorophyll fluorescence parameters. For dark adaptation, leaves were covered for 20 min. Initial fluorescence yield (F_0) was measured in leaves under dim light (< 0.05 μmol m^{-2} s^{-1}), following by a saturating pulse (3 000 μmol m^{-2} s^{-1}) to determine maximum fluorescence yield (F_m). Leaves were then exposed to actinic light (180 μmol m^{-2} s^{-1}) until the fluorescence was at steady state (F_s). Then the leaves were again illuminated with saturating pulse to determine F'_m. Maximum photochemical efficiency of photosystem (PS) 2 was estimated as $F_v/F_m = (F_m - F_0)/F_m$. Non-photochemical quenching was estimated as $NPQ = (F_m - F'_m)/F'_m$ (Bilger and Björkman 1990). Quantum efficiency of PS 2 photochemistry was estimated as $\Phi_{PS2} = (F'_m-F_s)/F'_m$ (Genty *et al*. 1989).

Electrolyte leakage, which reflected the membrane injury, was measured according to the method of Bajji *et al*. (2001). Briefly, the flag leaves (about 1 g) were cut and incubated in 20 cm^3 of distilled water at 25 °C for 1 h. After filtration, the leakage of electrolytes to the incubation medium was measured with conductivity meter (*ES-14E*, *Horiba*, Kyoto, Japan). Membrane lipid peroxidation was determined from content of malon-dialdehyde (MDA, Hong *et al*. 2000). About 0.5 g of the leaves was homogenized in 5 cm^3 of 10 % (m/v) trichloroacetic acid and the homogenate was centrifuged at 13 000 *g* for 15 min. The supernatant was mixed with an equal volume of thiobarbituric acid (0.5 % in 20 % trichloroacetic acid), and the mixture was boiled for 30 min at 100 °C, followed by centrifugation. Absorbance of the supernatant was measured at 532 nm and corrected for non-specific turbidity by subtracting A_{600}. MDA content was calculated using coefficient of absorbance of 155 M^{-1} cm^{-1}.

SOD activity was assayed by monitoring inhibition of photochemical reduction of nitroblue tetrazolium at 540 nm. The assay was performed with protein extracts from the leaves after homogenization in 0.005 M Tris-HCl buffer (pH 7.4) at 25 °C (Beyer and Fridovich 1987). One unit (U) of SOD activity was defined as the amount required to inhibit photoreduction of 0.6 mM nitroblue tetrazolium by 50 %. APX activity was assayed by recording the decrease in absorbance at 290 nm due to a decrease in ascorbic acid content (Nakano and Asada 1981). Reaction mixture (3 cm^3) contained 50 mM

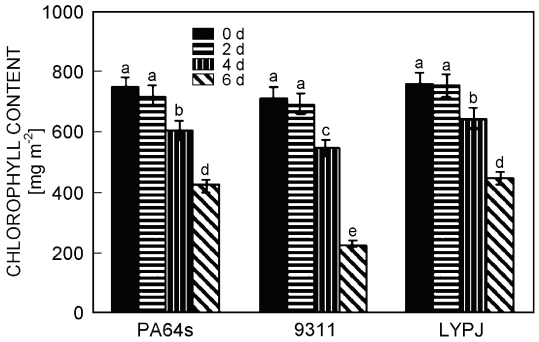

Fig. 1. Change of chlorophyll content of LYPJ and its parental cultivars under chilling stress. Means for each genotype that do not have a common letter are significantly different at $P < 0.05$ by Duncan's test.

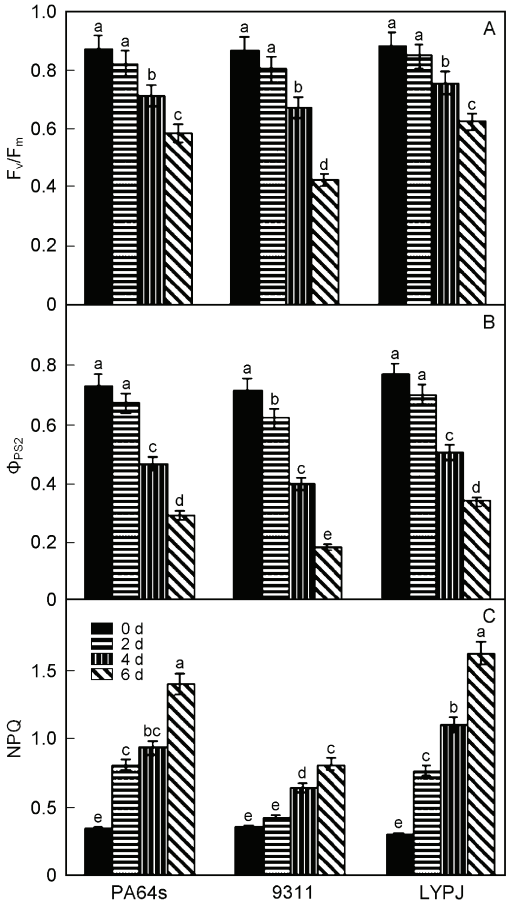

Fig. 2. Change of chlorophyll fluorescence in LYPJ and its parental cultivars under chilling stress. *A* - PS 2 maximum photochemical efficiency, F_v/F_m; *B* - the actual PS 2 efficiency, Φ_{PS2}; *C* - non photochemical quenching, NPQ; Means for each genotype that do not have a common letter are significantly different at $P < 0.05$ by Duncan's test.

potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM EDTA, and 1.5 mM H_2O_2 and 0.1 cm^3 enzyme extract. GR activity was assayed by the method of Smith *et al*. (1988). The reaction mixture contained 66.67 mM potassium phosphate buffer (pH 7.5), 0.333 mM EDTA, 0.5 mM 5,5-dithiobis-2-nitrobenzoic acid (DTNB) in 0.01 M potassium phosphate buffer (pH 7.5), 0.0667 mM NADPH, 0.1 cm^3 enzyme extract and distilled water to make up a final volume of 2.9 cm^3. Reaction was initiated by adding 0.667 mM oxidized glutathione. The increase in absorbance at 412 nm was recorded at 25 °C over a period of 5 min. The protein concentration was determined according to Bradford's method using bovine serum albumin as standard (1976).

One-way analysis of variance was performed using the *SPSS 10.0* computer package (*SPSS*, Chicago, USA) for all sets of data, and means were compared using Duncan's multiple comparison test at $P < 0.05$.

Chlorophyll content of three rice cultivars did not show significant differences before chilling (Fig. 1). However, the chlorophyll contents of plants chilled for 6-d was lower than that before chilling and the hybrid LYPJ was less affected than cv. 9311. F_v/F_m and Φ_{PS2} in the flag leaves also declined significantly during chilling stress while NPQ increased significantly and LYPJ and cv. PA64s were less affected than cv. 9311 (Fig. 2).

Exposure to low temperature for 6-d significantly increased the electrolyte leakage and MDA content (Fig. 3). After applying 15 °C for 6-d, both PA64s and 9311 showed higher values than LYPJ but, the difference of electrolyte leakage in LYPJ and PA64s was not significant ($P > 0.05$).

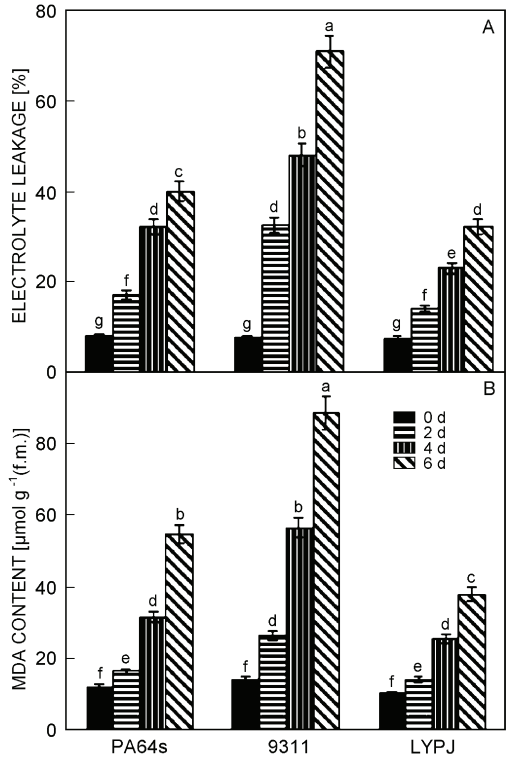

Fig. 3. Change of electrolyte leakage and MDA content in LYPJ and its parental cultivars under chilling stress. *A* - electrolyte leakage; *B* - malondialdehyde content, MDA; Means for each genotype that do not have a common letter are significantly different at $P < 0.05$ by Duncan's test.

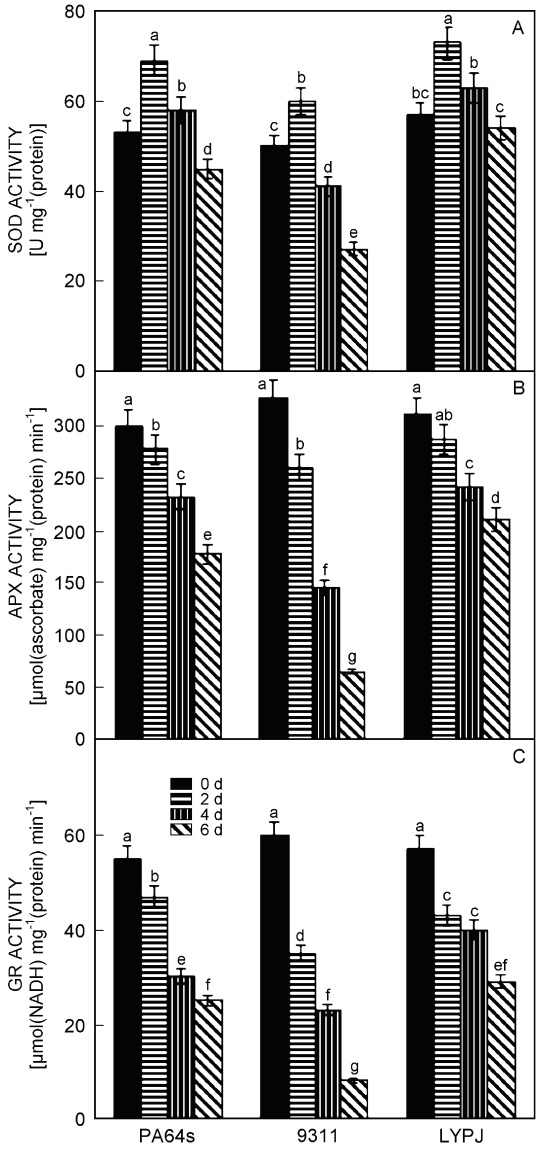

Fig. 4. Change of activities of enzymes scavenging ROS in LYPJ and its parental cultivars under chilling stress. *A* - superoxide dismutase, SOD; *B* - ascorbate peroxidase, APX; *C* - glutathione reductase, GR; Means for each genotype that do not have a common letter are significantly different at $P < 0.05$ by Duncan's test.

SOD activity in three rice cultivars increased and reached the peak at 2-d after chilling treatment, and then decreased slowly. SOD activity in the hybrid rice LYPJ increased faster than in its parents but the difference in relation PA64s was not significant (Fig. 4*A*). APX, and GR activities of chilled plants were lower than before chilling and LYPJ was less affected than 9311 (Fig. 4*B*,*C*).

It is a well documented fact that the function of a

photosynthetic apparatus is sensitive to several environmental stresses, such as high and low temperature stress, and salinity stress. PS 2 appears to be preferentially affected by chilling stress (Bolhàr-Nordenkampf and Öquist 1993, Agati *et al.* 1996, Bertamini *et al*. 2007). Chilling stress reduced the F_v/F_m and Φ_{PS2}, indicating that an important portion of the PS 2 reaction centre was damaged. The decrease of F_v/F_m and Φ_{PS2} in hybrid rice LYPJ was less than those of its parental cultivar under chilling stress (Fig. 2*A,B*). Thermal dissipation plays an important role in preventing over-reduction of PS 2 electron acceptors (Anderson *et al*. 1997, Gilmore 1997). In this study, NPQ of the three rice cultivars increased gradually during chilling stress (Fig. 2*C*), indicating a significant increase of thermal dissipation compensating the reduced photochemical dissipation.

Increased electrolyte leakage under chilling stress reflected the damage to the plasmalemma and LYPJ was less affected than its parental cultivars (Fig. 3*A*). These results were consistent to those of Morsy *et al*. (2007) who also found that chilling stress increased electrolyte leakage in rice. MDA content reflects the level of the membrane lipid peroxidation resulting from oxidative stress (Dhindsa *et al*. 1981). The results in this study indicated that the membrane integrity in the hybrid rice LYPJ was less injured by chilling stress than that of its parental cultivars. One possible mechanism contributing to lower electrolyte leakage and MDA content in the hybrid rice LYPJ could be that excessive light energy can be thermally dissipated leading to less ROS production. Another possible mechanism could be the high efficiency with which LYPJ scavenged the ROS (Amor *et al*. 2000) due to higher activities of SOD, APX and GR (Fig. 4 and Sairam *et al*. 2005, Dai *et al*. 2009, Wang *et al*. 2009).

In conclusion, hybrid rice LYPJ was more tolerant to chilling stress than its parental cultivars, but no or slightly significant differences were found between LYPJ and maternal cultivar PA64s under chilling stress. Those results demonstrated that adaptation to chilling stress in super-hybrid rice LYPJ might be adopted from its maternal cultivar.

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