BIOLOGIA PLANTARUM 50 (4): 597-602, 2006

Effect of water stress on photosystem 2 in two wheat cultivars

W.-J. LIU*, S. YUAN*, N.-H. ZHANG*, T. LEI*, H.-G. DUAN**, H.-G. LIANG* and H.-H. LIN*[1]

*Key Laboratory of Bio-resources and Eco-environment, Ministry of Education, College of Life Science, Sichuan University, Chengdu 610064, Sichuan, China**
*Department of Chemistry and Life Science, Neijiang Teachers College, Neijiang 641112, Sichuan, China***

Abstract

The effects of osmotic dehydration on the photochemical activity, gene transcription, and protein content of photosystem 2 (PS 2) in leaves of two wheat (*Triticum aestivum* L.) cultivars Miannnong No. 4 and No. 5 were studied. Roots of both cultivars were submerged into polyethylene glycol (PEG) solutions with an osmotic potential of -0.5 MPa for 0, 24, 48, and 72 h. Relative water content (RWC) decreased markedly after 48 and 72 h. Simultaneously, marked increase in electrolyte leakage, decrease in contents of chlorophylls (Chl) *a* and *b*, and inhibition in PS 2 activity were observed. Northern hybridization indicated that progressive water stress remarkably reduced contents of the chloroplast gene *psbA* and *psbD* and nuclear gene *cab* transcripts. Urea-SDS-PAGE and Western blotting analysis showed that the contents of major PS 2 proteins, including the D1 and D2 proteins in the PS 2 reaction centre (RC) and the light-harvesting Chl *a/b*-protein complex (LHC 2) in periphery, declined with increasing water stress. Miannong No. 5 had less destroyed plasma membranes and higher RWC, Chl contents, and PS 2 activity during water stress than Miannong No. 4, which suggested its better drought resistance. The significant difference in steady state contents of LHC 2 proteins of two cultivars can be mainly attributed to the marked difference in transcript level of *cab* gene, which indicated that LHC 2 proteins protect PS 2 RC.

Additional key words: chlorophylls *a* and *b*, DCIP reduction, D1 and D2 proteins, electrolyte leakage of cell membrane, gene expression, LHC 2, Northern blot, polyacrylamide gel electrophoresis, *Triticum aestivum*, Western blot.

Introduction

Drought being an important limitation for plant impairs severely growth, crop yield and various morphological, anatomical, physiological and biochemical processes (Egert and Tevini 2002, Upadhyaya and Panda 2004). During water stress, plants experience a number of metabolic changes, including changes in protein synthesis (He *et al*. 1999), alterations in gene expression (Skeriver and Mundy 1990, Bray 1993), decline of protein and chlorophyll contents (Hsu and Kao 2003), and production of reactive oxygen species (Upadhyaya and Panda 2004). Photosynthesis is sensitive to water stress and limitation of photosynthetic carbon metabolism has been analyzed in certain crop plants (Griffiths and Parry 2002, Lawlor 2002). Water deficit in leaves results in the reduction of net photosynthetic rate, which to some extent can be attributed to the impairment of the primary photosynthetic machinery (Kaiser 1987).

Photosystem (PS) 2, the pigment-protein complex in the thylakoid membranes, plays an important role in the photosynthetic responses to environmental stresses in higher plants (Baker 1991). Experiments on algae and isolated chloroplasts showed that PS 1 and PS 2, particularly PS 2, are affected by water stress which leads to a lower electron transport efficiency (Wiltens *et al*. 1978). Blocking of electron transport on the donor and acceptor sides of PS 2 has also been reported in films of

Received 27 September 2004, *accepted* 14 September 2005.
Abbreviations: Chl - chlorophyll; DCIP - 2,6-dichlorphenol indophenol; LHC - light-harvesting protein complex; PAGE - polyacrylamide gel electrophoresis; PEG - polyethylene glycol; RC - reaction centre; RWC - relative water content; SDS - sodiumdodecyl sulphate.
Acknowledgments: We thank Prof. Wu Nai-Hu, Prof. L.-F. Yan and Dr. Y.-S. Zhu for providing *psb*A, *psb*D, and *cab* genes as probes, respectively. We are indebted to Prof. E.-M. Aro for providing the antibodies against D1 and D2 proteins, and Prof. N. Yamamoto for the antibodies of LHC 2. This work was supported by the by National Nature Science Foundation of China (30571119), the Program for New Century Excellent Talents in University, the Doctoral Foundation of the Ministry of Education (20040610015), and Sichuan Science and Technology Foundation (04ZQ026-036).
[1] Corresponding author; fax: (+86) 028 85412571, e-mail: honghuilin@hotmail.com

dried chloroplasts (Matorin *et al.* 1982). It is hypothesized that environmental stresses may affect the antenna size and quantum efficiency of PS 2, thereby affecting CO_2 assimilation (Genty *et al.* 1989, Baker 1991, Hao *et al.* 1999). Specific changes in the fluorescence induction pattern also suggest that water stress hampers PS 2 activity (Govindjee *et al.* 1981, Havaux *et al.* 1986). Although many studies on PS 2 have been done, the mechanism by which water stress affects its photosynthetic activity remains to be elucidated. The light-harvesting chlorophyll-protein complex 2 (LHC 2) and the reaction centre (RC) perform the key steps in the process of photosynthetic energy collection and conversion, and PS 2 can catalyze both the water oxidation process and photochemical charge separation. Considering the loss of PS 2 activity during water stress is closely correlated with the steady state contents of these primary functional proteins, water stress influences their metabolism (Yuan *et al.* 2005). However, there have been no integrated studies on the metabolic changes of major functional proteins and the corresponding transcripts of PS 2 with progressive water stress.

In our study, two wheat cultivars with different drought resistance estimated by determining relative water content (RWC), electrolyte leakage, chlorophyll (Chl) contents, and activity of PS 2 were used to investigate the changes from transcription and translation of genes encoding major functional proteins to photosynthetic activity of PS 2 under progressive water stress.

Materials and methods

Plant growth and stress treatments: Seeds of two wheat cultivars (*Triticum aestivum* L. cv. Miannnong No. 4 and Miannnong No. 5) were surface-sterilized with 1 % NaClO for 10 min. The germinated seeds were planted in sterilized sand and grown for 10 d at 25 °C under a 12-h photoperiod and photosynthetic photon flux density (PPFD) of 100 μmol m^{-2} s^{-1}. For stress treatment, seedlings were carefully removed from the sand, washed with tap water, and dried briefly with paper towels to remove surface water. Water stress was initiated by submerging the roots into PEG solutions with an osmotic potential of -0.5 MPa in beakers. Control seedlings were grown in water and all samples were treated for 0, 24, 48, and 72 h under the above irradiance and temperature. The degree of water stress was characterized by RWC, which was determined as the ratio of (fresh mass - dry mass)/(water-saturated mass - dry mass).

Electrolyte leakage was measured according to Szalai *et al.* (1996) with some modifications: after measuring the electrical conductivity, the wheat samples were boiled at 100 °C for 15 min to achieve 100 % electrolyte leakage. The relative conductivity of plasma membranes was measured according to the radio of electrical conductivity before and after boiling.

The contents of Chl *a* and *b* were determined according to Lichtenthaler and Wellburn (1983).

Preparation of chloroplasts and thylakoids: Intact chloroplasts were prepared from wheat leaves according to Baishnab *et al.* (1980). Highly active thylakoids were prepared according to Kuwabare and Murata (1982) with some modifications: wheat leaves were mashed by a triturator in K_1 isolation solution (100 mM sucrose, 200 mM NaCl, 50 mM phosphate buffer, pH 7.4) for 40 s, filtered through two layers of cheesecloth, and the filtrates were centrifuged at 3 000 *g* for 10 min. The deposits were centrifuged at 500 *g* for 60 s after suspending with K_1, then the supernatant fluids were centrifuged at 3 000 *g* for 10 min, and the thylakoids were isolated after suspending with K_2 isolation solution (300 mM sucrose, 500 mM NaCl, 50 mM phosphate buffer, pH 6.9).

Determination of PS 2 photochemical activity: 2,6-dichlorophenol indophenol (DCIP) photoreduction was determined (*TU-1800* spectrophotometer, *P-general Limited Company*, Beijing, China) according to Tang and Satch (1985). Components of the reaction mixture were 50 mM Mes-NaOH (pH 7.5), 10 mM NaCl, 60 μM DCIP, 2 mM $MgCl_2$, and 20 μg Chl cm^{-3} intact chloroplasts.

SDS-PAGE and Western blot analysis: According to the method of Laemmli (1970), equal amounts of thylakoid membrane proteins were applied on SDS-PAGE containing 6 M urea. Stacking and resolving gels were 13.75 and 5.00 %, respectively. For analyzing the changes in polypeptide composition of thylakoid complex, gels were stained with Coomassie Blue R-250.

For Western blotting, electrophoresed proteins were immediately electrotransferred onto nitrocellulose membrane according to Sambrook *et al.* (1989). Then antisera to the D1, D2 (provided by E.-M. Aro), and LHC 2 proteins (provided by N. Yamamoto) were applied. The signals were revealed by using secondary antibodies of alkaline phosphatase goat anti-rabbit IgG. Semi-quantitative data on the contents of proteins were obtained by densitometric scanning of signal intensity of the Western blots.

Preparation of total RNA and Northern blot hybridization: Total RNA was extracted from leaves according to Zhang *et al.* (2004). For Northern blot analysis, equal amounts of total RNA (20 μg) were separated on formaldehyde agarose gels, then RNA was transferred to nitrocellulose filters (*Boehringer Lannheim*

Company, Dassel, Germany) for subsequent probe hybridization according to Sambrook *et al.* (1989). DNA fragments of the *psbA* gene (provided by Wu Nai-Hu), *psbD* gene (provided by Yan Long-Fei), and *cab* gene (provided by Zhu Yu-Sheng) were used as probes for RNA blot analyses. Semi-quantitative changes in the steady state of transcripts were obtained by scanning signal intensity of individual RNA blots.

Results

When roots of two wheat cultivar seedlings were immerged into PEG solution with osmotic potential -0.5 MPa for 0, 24, 48, and 72 h, leaf relative water content (RWC) decreased gradually: after 24 h, decrease in RWC was 5.66 and 3.62 %, respectively, in wheat cultivars, relative to control (0 h). However, at 48 and 72 h, RWC of Miannong No. 4 was reduced by 23.82 and 45.13 %, and that of Miannong No. 5 by 16.73 and 38.09 %, respectively (Fig. 1*A*).

The electrolyte leakage of both wheat cultivars was affected slightly at mild stress, while it increased dramatically at moderate and severe water stress. The electrolyte leakage of Miannong No. 4 increased 1.68-, 3.02-, and 4.07-fold after 24, 48, and 72 h of stress, respectively, and that of Miannong No. 5 increased 1.62-, 2.37-, and 3.40-fold (Fig. 1*B*). Hence the structure and function of cell membrane system of wheat was damaged seriously by water stress.

The contents of Chl *a* and *b* (Fig. 1*C*, *D*) in leaves of both cultivars were reduced during water stress. Chl *a*, content of Miannong No. 4 was reduced by 12.51, 33.69 and 41.87 %, and that of Miannong No. 5 by 4.45, 19.90, and 25.17 % at 24, 48, and 72 h stress. The contents of Chl *b* of the two wheat cultivars declined by 27.96, 45.71, and 54.46 % (Miannong No. 4), and 4.52, 22.00 and 31.87 % (Miannong No. 5), respectively.

Activity of PS 2, measured as DCIP photoreduction, decreased by stress. During the first 24 h of dehydration, DCIP photoreduction of both wheat cultivars decreased slightly (6.40 and 4.17 %), but significant decrease was observed after 48 h of stress. At 48 and 72 h after stress, DCIP photoreduction of Miannong No. 4 declined by 72.00 and 64.00 %, and that of Miannong No. 5 declined by 79.17 and 72.92 %, respectively (Fig. 1*E*).

Contents of proteins with molecular mass from 23 to 47 kDa declined markedly as water stress developed, including peripheric antenna complex LHC 2 (CP24, CP26, and CP29) and core antenna CP43 and CP47. In contrast, the contents of polypeptides with large molecular mass (such as Chl-binding proteins of PS 1) showed only a slight decrease. Obviously, PS 2 is subjected to more severe damage than PS 1 at progressive water stress.

The water stress-induced changes in selected proteins was measured by Western blot analysis with specific antibodies. The target proteins were D1 and D2 proteins suggested to form the reaction centre (RC) of PS 2 and LHC 2 aggregated in outside of the PS 2. We found a

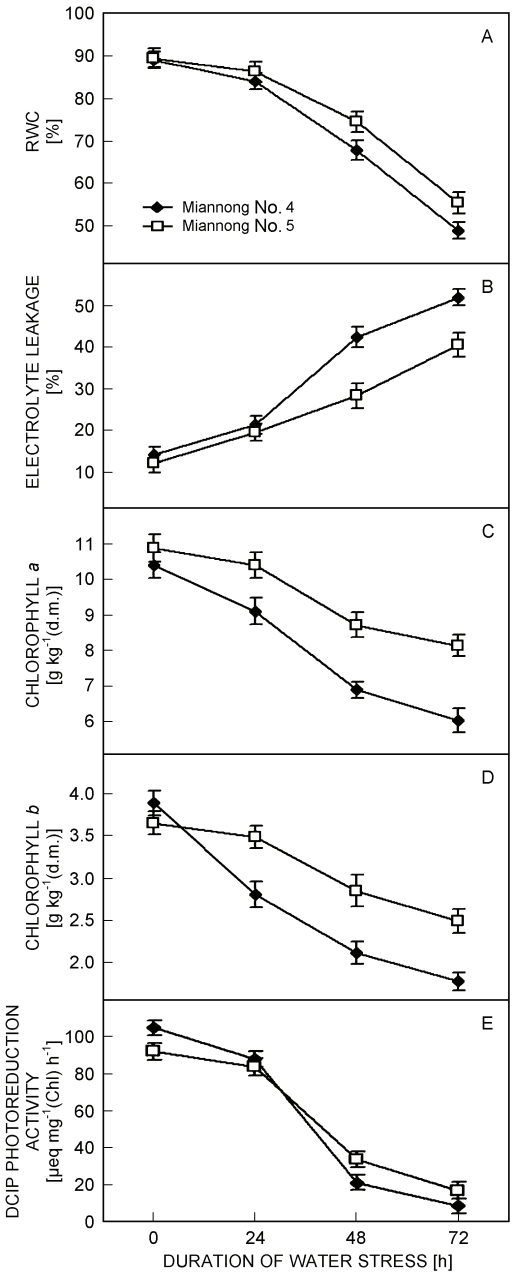

Fig. 1. Effect of water stress on leaf relative water content (RWC), electrolyte leakage of leaf cell membranes, contents of chlorophyll *a* and *b*, and PS 2 photochemical function indicated by DCIP photoreduction. Two wheat cultivar seedlings were root-stressed in PEG solutions of -0.5 MPa osmotic potential. *Bars* represent standard deviations of 5 (*A*) and 3 (*B-E*) independent replications.

progressive decline in the contents of LHC 2 proteins with advancing water stress. In contrast, the contents of D1 and D2 proteins decreased slightly within the first 24 h and significantly at 48 and 72 h (Fig. 3). In Miannong No. 4, approximately 33.60, 24.50 and 22.80 % reductions were observed for the contents of D1, D2, and LHC 2 proteins at 72 h, respectively, and the corresponding data were 30.41, 14.31, and 15.72 % for Miannong No. 5.

To estimate changes in transcript levels of particular genes, equal amounts of cellular RNA were blotted for Northern hybridization. The autoradiographs following hybridization (Fig. 4) revealed that the transcript levels of all genes decreased markedly and progressively as water stress developed. During the whole period of water stress, in Miannong No. 4 the transcript levels of *psbA*, *psbD*, and *cab* genes decreased by 29.38, 58.30 and 61.80 %, and those of Miannong No. 5 by 25.52, 49.68, and 39.50 %, respectively.

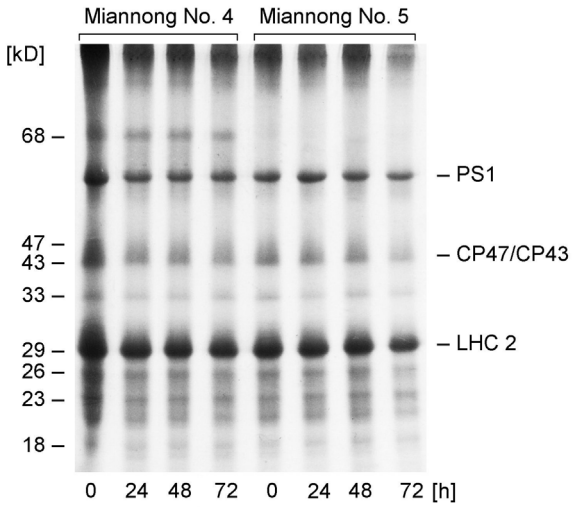

Fig. 2. Urea-SDS-PAGE of PS 2 polypeptides isolated from leaves stressed for 0, 24, 48, and 72 h. Equal amounts of thylakoid membrane proteins were loaded. The gel was stained with Coomassie Blue R-250.

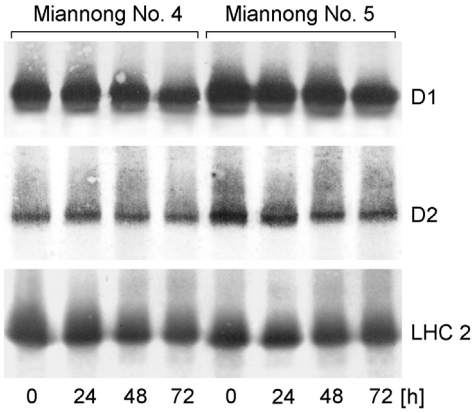

Fig. 3. Western blot analysis of steady state levels of the D1, D2, and LHC 2 proteins in PS 2 during water stress. Equal amounts of thylakoids from different stressed levels were separated by a urea-SDS-PAGE and electrotransferred to nitrocellulose membranes. The resulting blots were then allowed to immunoreact with the respective primary antibodies against D1, D2, and LHC 2 and with the secondary antibodies.

Discussion

According to the criteria for assessing the degree of water stress given by Hsiao (1973), stresses induced by PEG for 24, 48, and 72 h represented mild, moderate, and severe water stress, respectively. Earlier studies suggested high susceptibility of PS 2 to drought stress: even mild water stress could induce detectable decrease in PS 2 activity (Govindjee *et al*. 1981). However, more recent papers favour the view that PS 2 (Havaux 1992) or even the whole photosynthetic apparatus (Sharkey 1990) is quite robust in relation to water stress. Fluorescence studies and measurements of quantum yield of O_2 evolution performed on intact leaves (Cornic *et al*. 1991, Havaux 1992) show that PS 2 could tolerate high water deficit; mild stress did not substantially decrease PS 2 activity. In our experiments, osmotic stress did not significantly decrease photochemical activity of PS 2 until 48 h. This "robustness" of PS 2 was also shown by the analysis of the steady state contents of PS 2 polypeptides. Both urea-SDS-PAGE and Western blot analysis indicated that under mild water stress the composition and contents of PS 2 polypeptides in the thylakoid membrane did not markedly decrease.

RWC, Chl contents, and DCIP photoreduction of both wheat cultivars decreased, while electrolyte leakage of

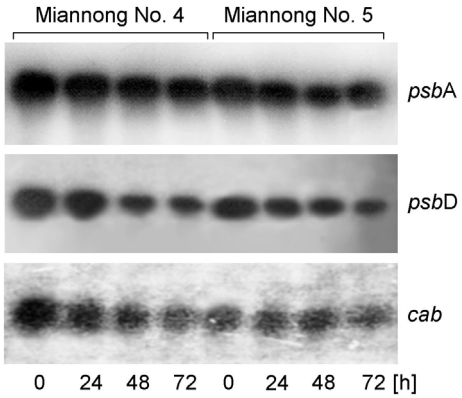

Fig. 4. Northern blot analysis of chloroplast gene *psb*A, *psb*D, and nuclear gene *cab* transcripts. Total RNA was prepared from stressed (24 to 72 h) and non-stressed (0 h) leaves and 20 μg portions were eletrophoretically separated. Following blotting, *psb*A, *psb*D, and *cab* transcripts were detected by hybridization with specific DNA probes.

plasma membranes increased under moderate and severe water stress. Hence water stress damaged the cell membranes, inhibited Chl synthesis (Pascaline *et al.* 2000), and affected photosynthetic efficiency.

Northern blot analyses with specific hybridization probes showed that the steady state transcript levels of *psbA*, *psbD*, and *cab* genes declined gradually during progressive water stress. For a particular gene, its transcript level may depend on three factors: *1*) transcriptional rate, *2*) mRNA stability, and *3*) template level. Our previous studies confirmed that no noticeable change in the template abundance of nuclear or chloroplast genes was observed under water stress (He *et al*. 1998). The progressive decreases in *psbA*, *psbD*, and *cab* gene steady state transcripts during water stress presumably can be attributed to lower gene transcriptional rates and/or changes of mRNA stability (Shu and Hong-Hui 2004, Yuan *et al.* 2005). This substantial decrease in gene transcript levels will certainly suppress translation of corresponding proteins (*i.e.* D1, D2, and LHC 2 proteins of PS 2). The urea-SDS-PAGE and Western analysis showed that the contents of major PS 2 functional polypeptides in stressed leaves decreased significantly. In addition, the results of *in vitro* translation of chloroplast RNAs (He *et al*. 1995) suggest that the synthesis of proteins from their mRNAs is barely affected by water stress in the intact chloroplasts. Therefore, the decreased steady state abundance of PS 2 proteins during water stress can be due to slowed translation and/or increased degradation.

Compared with Miannong No. 4, less damage on plasma membranes and slower decrease of Chl contents in Miannong No. 5 leaves during water stress were observed, which indicated that Miannong No. 5 has a better resistance to water deficit. Furthermore, the decline of *psbA*, *psbD*, and *cab* transcript levels in Miannong No. 5 was less than that in Miannong No. 4, which led to less decreased contents of the corresponding proteins (D1, D2, and LHC 2). Correspondingly, the DCIP photoreduction of Miannong No. 5 also declined more slowly.

After the 72 h of water stress, the decreasing amplitude of *cab* trascripts of Miannong No. 5 (39.5 %) was significantly less than that of Miannong No. 4 (61.8 %), which also caused the less decreased content of LHC 2 proteins in Miannong No. 5. Many studies suggest that the LHC 2 protein complex regulates the distribution of exciting energy between PS 1 and PS 2 by phosphorylation and de-phosphorylation (Allen 1995, 2003, Allen and Nilsson 1997). In the short-term water stress, LHC 2 protein complex regulates the transfer of excited energy to reaction centres through altering its conformation to avoid PS 2 RC to be destroyed (Hao *et al*. 1999). LHC 2 might protect PS 2 RC at water stress. In this way, the less decreased contents of D1 and D2 proteins of Miannong No. 5 can be explained. Thus the higher drought resistance of Miannong No. 5 is probably due to higher drought resistance of the *cab* gene. The PS 2 RC of Miannong No. 5 are likely protected better by higher LHC 2 content. Miannong No. 5 had a higher PS 2 photo-synthetic activity, as shown by the DCIP photoreduction.

In summary, our study primarily clarified the changes including gene transcription, protein metabolism, and photochemical function in progressive water stress. Water stress impaired photosynthetic activity of PS 2 by affecting the steady state contents of its primary functional protein complexes. Moreover, the comparison of two wheat cultivars with different drought resistance indicated that LHC 2 could be a crucial factor for the drought resistance of PS 2. However, it remains unclear whether the functions and activities of these proteins are weakened by altered structures or loosened correlation with other proteins during water stress.

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