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Stress-induced degradation of D1 protein and its photoprotection by DCPIP in isolated thylakoid membranes of barley leaf

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Abstract

Effects of various stress treatments such as NaCl, hydrogen peroxide, hydroxyl free radical, and high irradiance (HI, 1 000 μmol m^{-2} s^{-1}) on the photosystem (PS) 2 mediated electron transport rate and the degradation of D1 protein in the thylakoid membranes of barley were studied. The applied stresses caused significant reduction in the PS 2-mediated electron transport and a degradation of D1 protein that was highest during the HI-treatment. Presence of 2,6-dichlorophenol indophenol (DCPIP), which is an artificial electron acceptor from water, significantly minimizes the HI-induced deleterious effect on the PS 2-mediated electron transport rate, disarrangement of PS machinery, and degradation of the D1 protein. HI in the absence of an acceptor resulted in production of reactive oxygen species due to electron transfer to oxygen.

Additional key words: DCPIP, electron transport rate, *Hordeum vulgare,* photoinhibition.

Introduction

Photosynthetic organisms contain regulatory mechanisms of the photosynthetic apparatus, which participate in the dissipation of excess excitation energy and protect it against photoinhibition (Powles 1984). Photosystem (PS) 2 is the multi-subunit complex consisting of more than 30 polypeptides in thylakoid membranes of chloroplasts, and is responsible for the water oxidation reaction of photosynthesis. One of the most important features of PS 2 is its susceptibility to various environmental stresses. High irradiance (HI) and oxidative stress cause significant damage to PS 2. Damage to D1 protein, its cleavage and degradation, and re-assembly of the D1 protein and other subunits of PS 2 during the stresses are fundamental processes (Henmi *et al.* 2003). The D1 protein of PS 2 reaction centre is a target of photon-induced damage to the PS 2 complex and increasing irradiance accelerates the turnover of D1 protein (Aro *et al*. 1993). During proteolysis of damaged D1 protein and *de novo* synthesis of a new copy of D1, the repair cycle of PS 2 involves several other reactions, including post-translational processing (Baena-Gonzalez and Aro 2002).

NaCl treatment of isolated PS 2 membranes released PsbP (oxygen evolving complex, OEC23) and PsbQ (OEC17) completely of PS 2 membranes from spinach (Enami *et al*. 1989), and led to dissociation of extrinsic proteins (OEC33, OEC23, and OEC17) and degradation of other PS 2 polypeptides from *Euglena gracilis* (Suzuki *et al*. 2004). Reactive oxygen species (ROS) generated inside PS 2 under irradiation triggered the turnover of the D1 protein (Prasil *et al*. 1992), damaging the OEC subunits in isolated thylakoid and PS 2 preparations (Henmi *et al*. 2004). Mattoo *et al*. (1984) reported that the D1 protein had the highest turnover rate of all proteins in the thylakoid membrane under irradiation. Methyl viologen acts as an inducer of photo-oxidative stress

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Abbreviations: DCPIP - 2,6-dichlorphenol indophenol; DPC - 1,4-diphenyl carbazide; DTT - dithiothreitol; HI - high irradiance; LDS - lithium dodecyl sulfate; OEC - oxygen evolving complexes; PS - photosystem; ROS - reactive oxygen species

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(Donahue *et al*. 1997, Mano *et al*. 2001) and can induce the photoreduction of dioxygen (O_2) by accepting electrons from the iron-sulfur cluster Fe-S_A/Fe-S_B of PS 1, and thereby accelerates the production of superoxide radical ($O_2^{\bullet-}$) and H_2O_2 (Fujii *et al*. 1990). When catalytic free metals, *e.g*., Fe(II), Cu(I) and Mn(II), exist near the production site of $O_2^{\bullet-}$and H_2O_2, hydroxyl radical ($OH^{\bullet}$), the most toxic form of ROS, is produced from two ROS *via* Fenton reaction (Halliwell and Gutteridge 1984, Stadtman 1993).

Effect of various abiotic stresses such as; iron deficiency on electron transfer rate in *Pisum sativum* chloroplast (Muthuchelian *et al*. 2001), HI effect on PS 2 activity and PS 2 polypeptides in tobacco (Hofman *et al*. 2002), water stress effect on PS 2 activity as well as PS 2 polypeptides in wheat (Liu *et al*. 2006), and NaCl effect on protein profile in *Cajanus cajan* (Bishnoi *et al*. 2006) has been previously reported. In the present study we isolated intact chloroplasts and thylakoid membranes from barley leaves and compared the effects of various stresses such as salt (NaCl), hydrogen peroxide (H_2O_2), hydroxyl free radical ($OH^{\bullet}$), and high irradiance (HI, 1 000 μmol m^{-2} s^{-1}) on the PS 2-mediated electron transfer rate and on the degradation of thylakoid membrane polypeptides (particularly D1 protein). H_2O_2 (Bradley *et al.* 1991) as well as ROS could be directly produced by excited PS 2 in photoinhibitory conditions that trigger the turnover of the D1 protein (Prasil *et al*. 1992; Aro *et al*. 1993). HI irradiation of thylakoid membranes in the absence of an acceptor results in oxygen accepting electrons and production of ROS. The effect of 2,6-dichlorphenol indophenol (DCPIP), which is a Q_B site of electron acceptor and is generally used for the measurement of PS 2 activity and oxygen evolution, is well known (Maslenkova *et al*. 1995, Miyao *et al*. 1995, Henmi *et al*. 2004, Novakova *et al*. 2004, Porta *et al*. 2004). However, the effect of DCPIP on the thylakoid membrane polypeptides during photoinhibitory irradiation is poorly described. Therefore, we compared the effects of exogenous application of H_2O_2 and ROS ($OH^{\bullet}$), and HI-induced and endogenously produced ROS on the isolated thylakoid membranes. We also studied the effect of DCPIP that protects thylakoid membrane polypeptides (particularly D1 protein) by preventing oxygen action as an electron acceptor.

Materials and methods

Plants: Seeds of barley (*Hordeum vulgare* L. cv. Chabssal) were purchased from a farmer in Korea. The seedlings were germinated in the experimental field of Chonbuk National University, Chonju, South Korea. One-month-old seedlings, which were grown in pots, were then transferred to the laboratory and acclimated at low irradiation (20 μmol m^{-2} s^{-1}) for 1 week.

Intact chloroplasts were isolated according to the method of Busogoro *et al*. (2004), with some modifications. Barley leaves (18 g) were rinsed with distilled water, cut into small pieces, and homogenized in 35 cm^3 of isolation buffer (100 cm^3 of isolation buffer contained 330 mM sorbitol, 0.4 mM KCl, 0.5 mM $CaCl_2 . 2 H_2O$, and 2 mM Hepes, pH 7.6) and maintained continuously at 4 °C. The homogenate was filtered through 8 layers of *Miracloth*. The filtrate was centrifuged (centrifuge from *Hanil Science Industrial Co.,* Incheon, South Korea) at 100 *g* for 90 s. The resultant supernatant was re-centrifuged at 2 000 *g* for 5 min. The pellet obtained after this centrifugation step was re-suspended in cold isolation buffer with a total volume of 3.5 cm^3. Crude chloroplast suspension was carefully layered onto an equal volume (3.5 cm^3) of 35 % *Percol* prepared in isolation buffer, and centrifuged at 250 *g* for 5 min. The middle green layer, containing intact chloroplasts, was harvested and transferred to cold isolation buffer. This suspension was centrifuged at 2 000 *g* for 5 min and the supernatant was discarded. The pellet containing intact chloroplasts was resuspended in cold isolation buffer at a final concentration of 1.9×10^8 chloroplasts cm^{-3}. Total number of chloroplast was counted using hemacytometer (*Fisher Scientific Co.,* Pittsburg, USA) under a *Kyowa* microscope (*Kyowa Optical Co.,* Tokyo, Japan).

Thylakoid membranes were isolated by gentle sonication of intact chloroplasts. The sonicated suspension was centrifuged to pellet the thylakoid membranes (Smith *et al*. 2000). Required intact chloroplast suspension (5.7×10^7 chloroplasts) was further diluted in isolation buffer with the total volume of 35 cm^3 and sonicated (10 rounds for 180 s with short pulses at an amplitude of 50 % duty cycle) in a *Vibra-cell* microtip sonicator (*Sonics and Materials,* Danbury, CT, USA). During the sonication a complete disruption of chloroplasts and removal of thylakoid membranes in the suspension was observed with the optical microscope. To avoid the heat effect during sonication, temperature of the chloroplast suspension was maintained at 4 °C. Resultant thylakoid suspension was centrifuged for 60 min at 18 000 *g* and 4 °C. The pellet (thylakoid membranes) was re-suspended in a medium containing 50 mM MES-NaOH, pH 6.5, and 25 % glycerol at 0.28 mg cm^{-3} Chl *a,* and stored at -70 °C. Chlorophyll amount was determined by the method of Wellburn (1994).

Stress treatment: To compare the effects of various stresses, thylakoid membrane suspension (0.18 mg cm^{-3} Chl *a*) was incubated with 1 M NaCl, 3 mM H_2O_2, $OH^{\bullet}$

and HI of 1 000 μmol m^{-2} s^{-1} using a 150 W HQI lamp, and the combination of 1 mM DCPIP and HI treatment (HI + DCPIP) for 40 min. For the $OH^{\bullet}$ treatment, thylakoid membranes were incubated with 3 mM H_2O_2 and 1 mM $FeSO_4$ at 25 °C for 40 min. The suspension was then supplemented with 0.1 mg cm^{-3} catalase and 2 mM EDTA to stop the Fenton reaction as described by Henmi *et al.* (2004). To study the further time-course of protective effect of DCPIP under HI, thylakoid membrane suspension [0.18 mg (Chl *a*) cm^{-3}] was again incubated for 20, 40, and 60 min under HI and also at combination of 1 mM DCPIP and HI (HI+DCPIP). After incubation, the suspension was centrifuged for 60 min at 18 000 *g* and 4 °C.

Electron transport rate: The PS 2 mediated electron transport activities in terms of DCPIP photoreduction were measured in thylakoid membranes after the stress treatment following the procedures of Prasad *et al*. (1991) and Parida *et al*. (2003).

SDS-urea-PAGE: The resultant pellet containing thylakoid membrane proteins was dissolved in 0.036 cm^3 of denaturing buffer [5.2 % lithium dodecyl sulfate (LDS), 172 mM Tris-HCl, pH 8.0], 40 mM DTT, 0.5 M sucrose, 0.01 % Coomassie Brilliant Blue R-250) by a stirrer, and then heated for 1 min at 98 °C and centrifuged for 15 min at 12 000 g and 4 °C. The obtained supernatant was used for the sodium dodecyl sulphate-urea-polyacrylamide gel electrophoresis (SDS-urea-PAGE).

Electrophoresis conditions were similar to those of Kashino *et al.* (2001) in case of 20 % acrylamide gel containing 6 M urea. The dissolved sample containing thylakoid membrane polypeptides [0.25 mg (Chl *a*) cm^{-3}] was used for SDS-urea-PAGE. The gel was stained with 0.15 % Coomassie Brilliant Blue R-250 in a solution of 50 % methanol and 10 % acetic acid, and destained by the solution of 25 % methanol and 7.5 % acetic acid. It was scanned with a gel documentation system (*Core Bio System, Digital UV Transilluminator*).

Transfer of protein into membrane and Western blotting: Proteins were transferred to *Hybond-P PVDF* membrane (*Amersham Biosciences,* Little Chalfont, UK) overnight by passive transfer using blotting buffer [100 mM Tris, 192 mM glycine, 0.02 % (m/v) SDS and 5 % (v/v) methanol] as described by Kashino *et al*. (2001). Membrane was pre-wetted in 100 % (v/v) methanol, washed with distilled water for 5 min, and equilibrated in transfer buffer for at least 10 min before blotting. For immunoblotting, the membrane was incubated for 1 h at 25 °C with primary anti-*psbA* global polyclonal antibody raised against a peptide target conserved in PsbA/D1 protein from *Hen* (*AgriSera*, product number *AS01-016*). *Anti-Chicken IgG* peroxidase conjugate antibody developed in rabbit (*Sigma*, St. Louis, USA) was used as a secondary antibody. Band corresponding to D1 protein was detected with ECL Advance Western blotting detection kit (*Amersham Biosciences*) and exposed to autoradiography film (*Hyperfilm* ^{TM}*ECL*) in a cassette for 15 s and developed, while another autoradiography film was exposed for 30 min. Phosphate buffered saline containing 80 mM Na_2HPO_4, 20 mM NaH_2PO_4, 100 mM NaCl, pH 7.5, and 0.1 % (v/v) *Tween 20* was used as a base solution. Developed film was washed, dried, scanned with a *Gel Documentation System*, and quantified densitometrically.

Statistical analysis: Each parameter was repeated at least three times independently. Means and standard error were calculated from three replicates. When the interaction was significant, Duncan's multiple range test (DMRT) was used, $P = 0.05$.

Results

Effects of various stresses on the electron transfer rate and thylakoid membrane polypeptides: Significant decreases in the rate of DCPIP photoreduction was observed in thylakoid membranes after the various stress (NaCl, H_2O_2, $OH^{\bullet}$ and HI) treatments. Highest decrease was observed with NaCl and HI treatments (42 and 50 %, respectively) compared with control. In addition, PS 2 mediated electron transfer rate decreased more drastically under photoinhibitory HI compared with exogenously applied H_2O_2 and $OH^{\bullet}$ on the isolated thylakoid membranes (Fig. 1*A*). Exogenous electron donor (*i.e.* 1,4-diphenyl carbazide, DPC) was unable to restore the various stress-induced inhibitions of DCPIP photoreduction (Fig. 1*B*). On the other hand, PS 2-mediated electron transfer rate was significantly higher at HI + DCPIP, and also addition of exogenous electron donor (DPC) relatively restored stress-induced inhibition of DCPIP photoreduction, compared to HI stress alone. Thylakoid membrane polypeptides after various stress treatments were solubilized and subjected to SDS-urea-PAGE. Polypeptide profiles in control and various stress-treated samples were similar. However, the amounts of various polypeptides changed during the stress treatment. Significant decrease in the content of various polypeptides was observed during the HI treatment. Marginally more decreased amount of various polypeptides was recorded under HI treatment compared with exogenously applied H_2O_2 and $OH^{\bullet}$. However, the incubation of isolated thylakoid membranes with HI + DCPIP significantly reduced the HI-induced

photoinhibitory effect (data not shown). For the comparison of effects of various stresses on the content of D1 protein, with the use of primary anti-*psbA* global polyclonal antibody and *Anti-Chicken IgG* peroxidase as a secondary antibody, immunobloting was performed (Fig. 2*A*,*B*). It showed significant degradation of D1 protein under various stresses. Among them, incubation of thylakoid membranes under HI resulted in the highest degradation (83 %) of D1 protein compared with control. A significant decrease of D1 content was recorded with HI treatment as compared with exogenously applied H_2O_2 and $OH^{\bullet}$. Similarly, HI + DCPIP treatment resulted in significant protection from HI induced degradation of the D1 protein (Fig. 2).

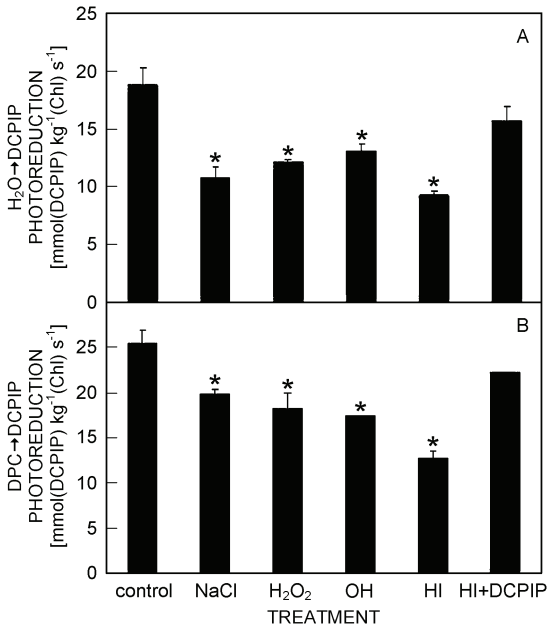

Fig. 1. Comparison of various stresses on the PS 2 mediated electron transport activities in isolated thylakoid membranes as monitored in terms of DCPIP photoreduction. The rates were measured after stress treatment in a reaction mixture at pH 7.0 as H_2O→DCPIP without any uncoupler and inhibitor (*A*), or DPC→DCPIP (*B*). Means of three independent measurements ± SE; * - means significantly different from the control. Control bar indicates measurements on untreated thylakoid membranes.

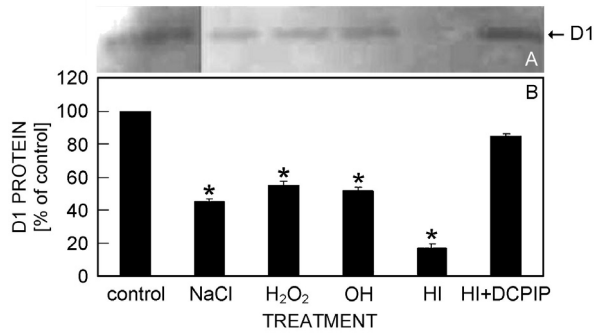

Fig. 2. *A* - Western blot of anti-*psbA* global polyclonal antibody raised against a peptide target conserved in *psbA*/D1 protein. *B* - Changes in the contents of 32-kDa D1 protein quantified densitometrically from immunoblots in relation to contents prior to treatment as control (100 %). Means of three independent measurements ± SE; * - means significantly different from the control. Control bar indicates measurements on untreated thylakoid membranes.

Time-course relation between HI and DCPIP on the electron transfer rate and thylakoid membrane polypeptide: To study the time-course relations between HI induced degradation and its protection by DCPIP, we further exposed the isolated thylakoid membranes to HI and HI + DCPIP for 20, 40, and 60 min. We observed a significant decrease of the PS 2-mediated electron transfer rate and degradation of thylakoid membrane polypeptides in correspondence with increasing the incubation period under photoinhibitory HI. Maximum significant decrease (62 %) was observed at 60 min after HI treatment compared with control (Fig. 3*A*). Similarly, exogenous electron donor (DPC) could not restore the HI-induced inhibition of DCPIP photoreduction that was

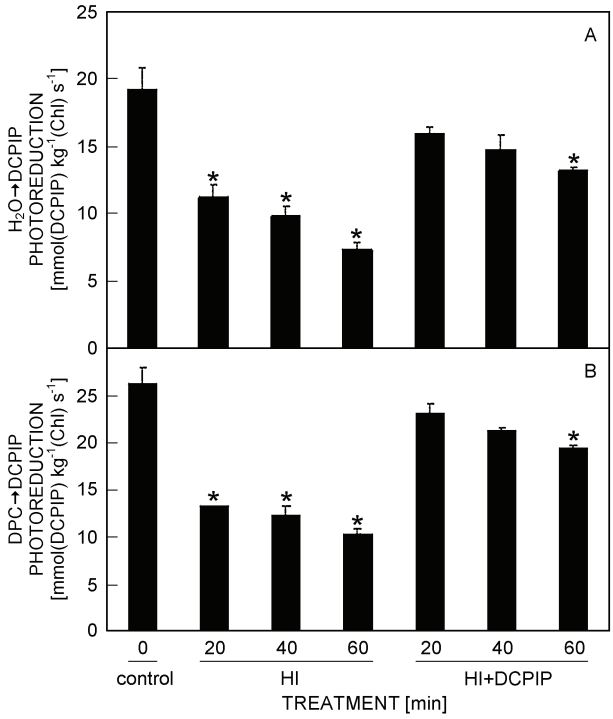

Fig. 3. Time course of the effect of high irradiance (HI, 1 000 µmol m^{-2} s^{-1}) without and with DCPIP on the thylakoid membranes of barley leaf. PS 2-mediated electron transport activity in isolated thylakoid membranes was monitored in terms of DCPIP photoreduction. The rate was measured after stress treatment in a reaction mixture at pH 7.0 as H_2O→DCPIP without any uncoupler and inhibitor (*A*), or DPC→DCPIP (*B*). Mean of three independent measurements ± SE; * - means significantly different from the control. Control bar indicates measurements on untreated thylakoid membranes.

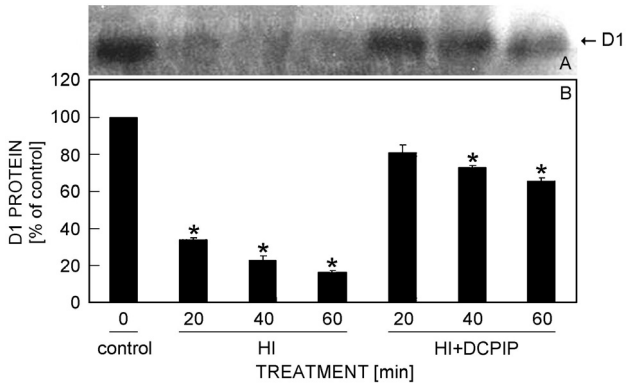

Fig. 4. *A* - Western blot of anti-*psbA* global polyclonal antibody raised against a peptide target conserved in *psbA*/D1 protein. *B* - Changes in the contents of 32-kDa D1 protein quantified densitometrically from immunoblots taking the amounts prior to treatment as control (100 %). Means of three independent measurements ± SE; * - means significantly different from the control. Control bar indicates measurements made on untreated thylakoid membranes.

marginally decreased with increasing the incubation period (Fig. 3*B*). Moreover, PS 2-mediated electron transfer rate was significantly higher at HI + DCPIP, and addition of exogenous electron donor (DPC) relatively restored stress-induced inhibition of DCPIP photoreduction, compared to HI stress alone (Fig. 3). Time-course of HI stress and HI + DCPIP stress treatments on the thylakoid membrane polypeptides was also studied: polypeptide profiles in control, HI, and HI + DCPIP treated samples were nearly similar. However, the amounts of various polypeptides changed apparently with increasing the duration of HI treatment. Incubation of isolated thylakoid membranes with HI + DCPIP significantly minimized the HI-induced photoinhibitory effect (data not shown). Similarly, immunobloting of the D1 protein indicated apparent (Fig. 4*A*) as well as highest degradation (84 %) during 60 min of HI treatment (Fig. 4*B*), while significant photoprotection was recorded in the presence of DCPIP (Fig. 4).

Discussion

For the functional integrity of photosystems and optimal photon energy harvesting, proper alignment of thylakoid membranes is essential. In the present study treatment by various stresses caused structural disarrangement within photosynthetic membranes, hampering the normal function of thylakoid membranes in the photon energy harvesting and utilization. To study the intensity of imposed stresses, we measured the PS 2-mediated electron transfer rate in terms of DCPIP photoreduction. Significant decreases in the rate of DCPIP photoreduction in thylakoid membranes after the various stress treatments (Fig. 1*A*), might be due to changes in the proper alignment of thylakoid membranes and also dissociation of some extrinsic proteins. NaCl-induced loss in PS 2-mediated electron transport activity has already been reported (Murata *et al.* 1992, Hernández *et al*. 1995, Tiwari *et al*. 1997, Allakhverdiev *et al*. 2000a,b). Irradiation of PS 2 membranes with strong white light decreased the PS 2 activity, monitored by the activity of DCIP photoreduction with a half-time of about 30 min (Henmi *et al*. 2004). HI treatment of the thylakoid membranes resulted in formation of H_2O_2 and $OH^{\bullet}$ that was reflected in decreased PS 2-mediated electron transfer rate. However, highest decrease (50 %) was recorded with HI treatments compared with control. PS 2-mediated electron transfer rate decreased more drastically under HI compared with exogenously applied H_2O_2 and $OH^{\bullet}$. Therefore, HI-induced endogenously generated ROS caused more deleterious effect in the decrease of PS 2-mediated electron transfer rate, compared with exogenously applied H_2O_2 and $OH^{\bullet}$ stresses. Similarly, DCPIP played important role in the protection of PS 2-mediated electron transfer rate. We also determined the effects of artificial electron donor (DPC) on inhibition of DCPIP photoreduction. Exogenous electron donor (DPC) was unable to restore the various stress-induced inhibitions of DCPIP photoreduction, suggesting that inhibition of electron transport rate might be either due to inactivation of the PS 2 reaction centre or to a change in membrane structure of the photosynthetic apparatus. Similar results were reported in studies of the inhibitory effects of NaCl on *Bruguiera parviflora* (Parida *et al*. 2003) or of nickel and zinc on barley chloroplasts (Tripathy and Mohanti 1980, Tripathi *et al*. 1981). On the other hand, in HI + DCPIP treatment, addition of DPC relatively restored stress induced inhibition of DCPIP photoreduction, compared to HI stress alone (Fig. 1*B*).

Although the SDS-PAGE of thylakoids revealed that the intensity of the many polypeptide bands significantly decreased in various stress (NaCl, H_2O_2, $OH^{\bullet}$, and HI) treated thylakoid membranes as compared to control. However, highest decrease was observed during HI-treatment. Similarly, marginal reduction in the amounts of various membrane polypeptides under HI-treatment compared with exogenously applied H_2O_2 and $OH^{\bullet}$, indicated that HI-induced endogenously generated ROS caused deleterious effect not only in the decrease of PS 2-mediated electron transfer rate, but also in reduction of contents of other thylakoid membrane polypeptides (data not shown). Immunoblotting of D1 protein indicated the apparent (Fig. 2*A*) as well as significant degradation of D1 protein under various stresses, while highest degradation was observed during HI treatment (Fig. 2*B*). The target site of various stresses may be the dissociation

of certain thylakoid polypeptides and HI induced the maximum effects. Our result supports the findings of Murata *et al*. (1992) and Suzuki *et al.* (2004) of significant reduction in the amounts of D1, D2, CP43, and CP47 polypeptides, and in completely release of OEC subunits (33, 23, and 17 kDa) from the PS 2 complexes, which play major role in oxygen evolution. The production of ROS inside PS 2 under irradiation triggers the turnover of the D1 protein (Prasil *et al*. 1992, Aro *et al*. 1993) and damages the OEC subunits in isolated thylakoid and PS 2 preparations (Henmi *et al*. 2004). We found a significant decrease of D1 content at HI-treatment compared with exogenously applied H_2O_2 and $OH^{\bullet}$ (Fig. 2). ROS influence the outcome of photo-damage primarily *via* inhibition of translation of the *psbA* gene, which encodes the precursor to D1 protein (Nishiyama *et al*. 2001), and the rate of photo-damage is proportional to irradiance. This relationship was not affected by oxidative stress (H_2O_2) (Allakhverdiev and Murata 2004). Similar to our results (Fig. 2), Hundal *et al*. (1990) described that during irradiation the amounts of the D1 protein and OEC33 in PS 2 membranes decreased. Significant protective effect on the degradation of various thylakoid membrane polypeptides in the protein profile (data not shown) as well as apparent and significant protective effect of D1 protein (Fig. 2) were observed during HI + DCPIP treatment compared to HI and other stresses. This indicates that incubation of thylakoid membranes under HI might have higher degradation capacity compared to other stresses imposed in the present study, and DCPIP plays important role in the protection of thylakoid membrane polypeptides (particularly D1 protein).

Time course analysis of HI-induced degradation and its protection by DCPIP indicated the significant decrease in the PS 2-mediated electron transfer rate corresponding with increase of the incubation period under photoinhibitory HI. In the present study (Fig. 3*A*), a significant decrease (62 %) of PS 2-mediated electron transport rate after 60 min of HI treatment compared with control indicated maximum disarrangement of thylakoid membranes and release of extrinsic proteins. Photo-reduction of DCPIP in PS 2-mediated electron transport rate and oxygen evolution have been reported (Maslenkova *et al*. 1995, Nováková *et al*. 2004, Porta *et al*. 2004), also, strong "white light" induced loss (50 % of about 30 min) in PS 2-mediated electron transport activity (Henmi *et al*. 2004). On the other hand, PS 2-mediated electron transfer rate was significantly higher at HI + DCPIP, and addition of exogenous electron donor (DPC) relatively restored stress-induced inhibition of DCPIP photoreduction, compared to HI stress alone (Fig. 3*B*). Thus DCPIP plays an important role in minimizing the HI-induced deleterious effect on the electron transport rate that might be either due to partial activation of the PS 2 reaction centre or to stabilization of the membrane structure of the photosynthetic machinery.

We found that incubation of isolated thylakoid membranes under HI resulted in significant degradation of thylakoid membrane polypeptides compared with control while significant photoprotection was recorded in the presence of DCPIP (data not shown). Immunoblotting of the D1 protein indicated significant degradation with increasing the irradiation period, which was highest (84 %) after 60 min of HI-treatment. Similar results have been reported in studies of Henmi *et al*. (2004) and Hundal *et al*. (1990) where the content of the D1 protein decreased in parallel with the HI-irradiation. In the present study a significant photoprotection of D1 protein was recorded during the HI + DCPIP treatment (Fig. 4). Hence HI in the absence of an acceptor results in production of oxygen accepting electrons and ROS. However, in the presence of DCPIP that works as an electron acceptor it protects D1 protein by preventing oxygen action as an acceptor. Although we performed the immunoblot of D1 protein only, our SDS-urea-PAGE profile (data not shown) indicates that DCPIP-induced photoprotection during photoinhibitory irradiation might be true for some other thylakoid membrane polypeptides. Hence, DCPIP probably plays an important role in the dissipation of excess excitation energy and protection of thylakoid membrane protein (particularly D1 protein) from HI-induced photoinhibitory effect.

In conclusion, various stresses caused significant reduction in the PS 2-mediated electron transport and degradation of D1 protein that was highest during the HI-treatment. DCPIP, which is an artificial acceptor of electron from water, significantly minimizes the HI-induced deleterious effect on electron transport rate, photosystem machinery, and degradation of D1 protein. During HI-treatment DCPIP works as an electron acceptor and protects from decrease in electron transport rate, disarrangement of photosystem machinery, and degradation of D1 protein by preventing oxygen action as an acceptor and producing ROS.

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Kimble, J.M., Rice, C.W., Reed, D., Mooney, S., Follett, R.F., Lal, R. (ed.): **Soil Carbon Management. Economic, Environmental and Societal Benefits.** - CRC Press, Taylor and Francis Group, Boca Raton - London - New York 2007. 268 pp. ISBN 13: 978-1-4200-4407-2.

The request to review this book was quite welcome to me. The title suggested that the content deals with carbon that definitely plays a far reaching role not only for plants but literally for the whole mankind. And soils are increasingly considered as the most precious value in nature. Furthermore, the subtitle of the book mentions the necessity to evaluate soil management also from the point of not only environment but also economic and environmental impact. And, last but not least, the editors represent well known experts not only in the very soil and carbon science but they are also engaged in formulating suggestions and measures needed to be realised in order to avoid economic crashes due to otherwise unavoidable changes of ecosystems and their functions.

I am aware that the introductory paragraph of this review simply repeats the book title. But it is my attempt to emphasize that the reader is really offered what the title of this book promises. Furthermore, the promise is fulfilled at a very high scientific standard. And this is not always the case of many publications. The Foreword was written by J. Moseley, farmer and former deputy secretary at the U.S. Department of Agriculture, and W. Richards, farmer and past chief of Soil Conservation service of the same Department. Detailed information about the editors and a list of contributors comes next. All the contributors are from the USA.

The contributions are divided into 3 sections as follows. Section I. "Overview, Policy, and Economics" contains 4 contributions (pp. 3 to 98) dealing with soil carbon management and its various values and benefits. Section II "On-Site Benefits" (pp. 99 to 164) with 4 contributions is devoted to particular conditions of cropland and grazing systems, organic farming practices, physical, chemical and biological soil properties. The largest Section III "Off-Site Benefits (pp. 165 to 262) includes 7 chapters. Seemingly, this section is relatively outside the direct soil management. Nevertheless, I find this part extremely valuable. The far reaching effects of erroneous soil management are too often overlooked and their impact neglected until a collapse in soil fertility bursts out. The contributions pay attention to soil erosion, wetlands, wildlife, flooding, surface water quality, urban lands, prairies, savannas and forests.

An Index terminates the book.

In the individual contributions many detailed results have been incorporated and lists of valuable references. However, I most appreciate the conclusions and warnings on the real thread of soil fertility destruction. Furthermore, the mutual relationship between soil properties and carbon sequestration is very well documented. And last but not least, the assessment of various soil managements takes into account not only the very soil properties, but also the economic, environmental and societal consequences.

Many examples and analyses included in the contributions are related to the conditions of the United States. But the conclusions and recommendations apply globally. If my extremely positive impression on the reviewed book attracts more readers – scholars, researchers and students, but also ecologists, agronomists and soil managers or decision makers, then my intention when writing this review is accomplished.

L. NÁTR (*Prague*)