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High irradiance induced changes of photosystem 2 in young and mature needles of cypress (*Cupressus sempervirens* L.)

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Abstract

Photoinhibition of photosynthesis was studied in young and mature detached sun needles of cypress under high irradiance (HI) of about 1 900 μmol m^{-2} s^{-1}. The degree of photoinhibition was determined by means of the ratio of variable to maximum chlorophyll (Chl) fluorescence (F_v/F_m) and electron transport measurements. Compared with the mature needles, the young needles, containing about half the amount of Chl *a*+*b* per unit area, exhibited a higher proportion of total carotenoids (Car) as xanthophyll cycle pigments and had an increased ratio of Car/Chl *a*+*b*. The potential efficiency of photosystem (PS) 2, F_v/F_m, markedly declined in HI-treated young needles without significant increase of F_0 level. In contrast, the F_v/F_m ratio declined with significant increase of F_0 level in mature needles. In isolated thylakoids, the rate of whole chain and PS2 activity markedly decreased in young HI-needles in comparison with mature needles. A smaller inhibition of PS1 activity was observed in both needles. In the subsequent dark incubation, fast recovery was found in both needle types that reached maximum PS2 efficiencies similar to those observed in non-photoinhibited needles. The artificial exogenous electron donors DPC, NH_2OH, and Mn^{2+} failed to restore the HI-induced loss of PS2 activity in mature needles, while DPC and NH_2OH significantly restored it in young needles. Hence, HI-inactivation was on the donor side of PS2 in young needles and on the acceptor side of PS2 in mature needles. Quantification of the PS2 reaction centre proteins D1 and 33 kDa protein of water splitting complex following HI-exposure of needles showed pronounced differences between young and mature needles. The large loss of PS2 activity in HI-needles was due to the marked loss of D1 protein of the PS2 reaction centre in mature needles and of the 33 kDa protein in young needles.

Additional key words: acclimation to irradiance; chlorophyll fluorescence; D1 protein; donor side; electron transport; photoinhibition; photosystem 2; proteins.

Introduction

Radiant energy is necessary to drive the process of photosynthesis; however, absorbed photons in excess of those required for the saturation of photosynthesis may cause its photoinhibition. Photoinhibition results from over-excitation of the photosynthetic apparatus (Powles 1984). Plants have developed a range of mechanisms to protect their photosynthetic machinery against over-excitation (Anderson *et al.* 1997). One such protective mechanism that has received recent attention is the thermal dissipation of the excess radiant energy in the light-harvesting antenna complexes of photosystem 2 (PS2) measured as non-photochemical quenching (for reviews see Demmig-Adams and Adams 1996, Gilmore 1997, Horton *et al.* 1999). Nevertheless, a major proportion of NPQ is the result of combined effects of the lumen acidification and de-epoxidation of violaxanthin (V) into zeaxanthin (Z) and antheraxanthin (A) in the xanthophyll cycle (Eskling *et al.* 1997, Gilmore 1997, Horton *et al.* 1999). A recent

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Abbreviations: A – antheraxanthin; Car – carotenoids; Chl – chlorophyll; DCBQ – 2,6-dichloro-*p*-benzoquinone; DCPIP – 2,6-dichlorophenol indophenol; DPC – diphenyl carbazide; F_0 – minimal fluorescence; F_m – maximum fluorescence; HI – high irradiance; MV – methyl viologen; PPFD, photosynthetic photon flux density; PS – photosystem; SDS-PAGE – sodium dodecylsulphate-polyacrylamide gel electrophoresis; RC – reaction centre; SiMo – silicomolybdate; TBS – Tris-buffered saline; V – violaxanthin; Z – zeaxanthin.
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hypothesis emphasizes the importance of the structural flexibility of the thylakoid membrane in controlling the energy dissipation (Horton *et al.* 1999). This mechanism relieves the excitation pressure on PS2 by converting excitation energy harmlessly into heat and thereby preventing the formation of damaging reactive oxygen species; it protects the photosynthetic apparatus against photodamage (Richter *et al.* 1990, Demmig-Adams and Adams 1996, Huner *et al.* 1998). De-epoxidation of V into Z *via* intermediate A is controlled primarily through the enzyme de-epoxidase (Yamamoto 1979) located in the thylakoid lumen **(**Hager 1969). The activity of the de-epoxidase is also co-regulated by the concentration of ascorbate and other essential co-substrates of the deepoxidase (Bratt *et al.* 1995).

Several investigators also view photoinhibition of photosynthesis as a process of stress-induced damage to PS2. This is because as a result of photoinhibition the D1 protein of PS2 reaction centre (RC) becomes degraded (Kyle *et al.* 1984, Prasil *et al.* 1992, Rintamaki *et al.* 1995). The down regulation of PS2 and thermal dissipation probably protects against high irradiance (HI) stress (Cleland *et al.* 1986, Eckert *et al.* 1991, Gilmore and Björkman 1994). The photoinactivation and impairment of electron transport occurs at the acceptor and donor sides of PS2, although inactivation of the acceptor side may be the main mechanism for the impairment of electron transport (Aro *et al.* 1993).

Studies of canopy sun leaves of several tree species of the tropical forest (Krause *et al.* 1995) showed a higher susceptibility to photoinhibition of young, light green leaves, as compared with corresponding mature, dark green leaves. Apparently, in the young leaves, a given irradiance results in high excitation of chlorophyll (Chl) due to their lower Chl content per unit leaf area. Thus, young leaves experience more severe HI stress than the mature ones**.** In the young tropical canopy leaves, a dominant fast phase, apparently related to epoxidation of Z, characterizes recovery from photoinhibition (Thiele *et al.* 1997).

The effect of photoinhibition, alone or interacting with other stresses, on the long-term consequences of carbon balance or productivity are illustrated by many authors (Flexas *et al.* 2001, Bertamini and Nedunchezhian 2002). This, as Powles (1984) noted, is in part because the recovery of plants from photoinhibition has received very little attention. A limited number of studies have shown that recovery takes at least several hours to occur (Ottander *et al.* 1995, Flexas *et al.* 2001, Bertamini and Nedunchezhian 2002). In fresh-water phytoplankton, for instance, recovery of photosynthesis occurred over 4–20 h, depending on the extent of inhibition (Belay 1981). Similarly, the recovery of photon yield of leaves of *Phaseolus vulgaris* took between 4 and 8 h (Powles *et al.* 1983).

The D1 protein of PS2 RC is a target of photon-induced damage to the PS2 complex; increasing irradiance accelerates the turnover of D1 protein (Aro *et al.* 1993). The hypothesis that degradation of D1 protein may regulate the functioning of the PS2 repair cycle under photoinhibitory conditions has arisen from experiments with higher plants acclimated to different growth irradiances. The low iradiance grown or shade plants are more susceptible to photoinhibition than HI or sun species (Öquist *et al.* 1992, Aro *et al.* 1993). This higher susceptibility is accompanied by slower degradation of D1 protein (Tyystjarvi *et al.* 1992, Aro *et al.* 1993). In addition to the proteolysis of damaged D1 protein and *de-novo* synthesis of a new copy of D1, the repair cycle of PS2 involves several other reactions, including post-translational processing and modification of the D1 protein, and ligation of the electron-transfer components (Flexas *et al.* 2001, Bertamini and Nedunchezhian 2002, Adir *et al.* 2003).

To our knowledge, nobody evaluated yet the importance of photoinhibition in cypress under controlled conditions. Therefore, the objective of our work was to compare the susceptibility to photoinhibition and the process of recovery in young and mature needles of cypress. We tested the effect of photoinhibition with respect to photosynthetic oxygen evolution and potential PS2 function by fluorescence. We also analyzed the amounts of D1 and 33 kDa proteins in relation to the functional properties of PS2 after photoinhibition. The significance of the capacity of the PS2 repair cycle for the protection against photoinhibition was analyzed by establishing rates of recovery from photoinhibition.

Materials and methods

Plants: Young and mature needles of cypress (*Cupressus sempervirens* L.) were collected from selected 20-year-old trees grown under field condition in Istituto Agrario di San Michele all' Adige, Italy. We harvested needle samples early in the morning, choosing sun-exposed light green, almost fully expanded young needles and dark-green, mature needles.

Photosynthetic pigments in leaf sections were analyzed by HPLC according to the method of Krause *et al.* (1995). The amount of Chl *a*+*b* per unit leaf area was determined according to Lichtenthaler (1987) after extraction of leaf segments with 80 % acetone in the presence of Na_2CO_3. We measured fractional absorbance (400–700 nm) of leaves as described by Krause *et al.* (1995).

Photoinhibition and recovery under controlled conditions: We placed detached needles into a controlled-environment chamber equipped with a 24 V/250 W metal-halide lamp (*H. Walz*, Effeltrich, Germany). The upper leaf surface was exposed to a photosynthetic photon flux density (PPFD) of 1 900 μmol m^{-2} s^{-1}. Air temperature

was 20 °C and relative humidity 66±5 %. A quantum sensor (*LI-Cor*, Lincoln, NE, USA) measured the PPFD. Leaf temperatures recorded with thermocouple attached to the lower surface were 27–29 °C. Detached young and mature needles after specified times of HI exposure were placed on moist filter paper in petri dishes. The needles were darkened for 5 min before the degree of photoinhibition was determined by fluorescence measurement. For recovery from photoinhibition the needles were kept in complete dark for 60 min.

Modulated Chl fluorescence was measured on needles using a *PAM 210* fluorometer (*H. Walz*, Effeltrich, Germany). Before the measurements, the needles were dark adapted for 30 min. F_0 was measured by switching on the modulated radiation of 0.6 kHz; PPFD was less than 0.1 μmol(photon) m^{-2} s^{-1} at the leaf surface. F_m was measured at 20 kHz with a 1-s pulse of 6 000 μmol(photon) m^{-2} s^{-1} of "white light".

Activities of photosynthetic electron transport: Thylakoid membranes were isolated from the needles as described by Berthold *et al.* (1981). Whole chain electron transport (H_2O→MV) and partial reactions of photosynthetic electron transport mediated by PS2 (H_2O→DCBQ; H_2O→SiMo) and PS1 ($DCPIPH_2$→MV) were measured as described by Nedunchezhian *et al.* (1997). Thylakoids were suspended at 10 g(Chl) m^{-3} in the assay medium containing 20 mM Tris-HCl, pH 7.5, 10 mM NaCl, 5 mM $MgCl_2$, 5 mM NH_4Cl, and 100 mM sucrose supplemented with 0.5 mM DCBQ and 0.2 mM SiMo.

DCPIP photoreduction rate was determined as the decrease in absorbance at 590 nm using a *Hitachi 557* spectrophotometer. The reaction mixture (3 cm^3) contained 20 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 10 mM NaCl, 100 mM sucrose, 0.1 mM DCPIP, and thylakoid membranes equivalent to 20 μg of Chl. Where mentioned, the concentration of $MnCl_2$, DPC, and NH_2OH were 5.0, 0.5, and 5.0 mM, respectively.

Immunological determination of thylakoid proteins was done by Western blotting. Thylakoids were solubilized in 5 % SDS, 15 % glycerine, 50 mM Tris-HCl (pH 6.8), and 2 % mercaptoethanol at room temperature for 30 min. The polypeptides were separated by SDS-PAGE as described by Laemmli (1970) and proteins were then transferred to nitrocellulose by electro-blotting for 3 h at 0.4 A. After saturation with 10 % milk powder in *Tris*-buffered saline (TBS) buffer (pH 7.5), the first antibody in 1 % gelatine was allowed to react overnight at room temperature. After washing with TBS containing 0.05 % *Tween-20*, the secondary antibody [*Anti-Rabbit IgG* (whole molecule), *Biotin Conjugate*, *Sigma*] was allowed to react in 1 % gelatine for 2 h. For detection of D1 protein a polyclonal antiserum against spinach D1 protein was used (kindly provided by Prof. I. Ohad, Jerusalum, Israel), and the antibody against the 33 kDa protein of the water-splitting system was a gift from Dr. Barbato, Padova, Italy. The densitometric analysis of Western blots was performed with a *Bio-Image* analyser (*Millipore Corporation*, Michigan, USA).

Results

Changes in pigments (Table 1): The Chl *a*+*b* content per unit area in young needles was about 53 % of that in mature needles, but the Chl *a/b* ratio was not significantly different between the two leaf types (Table 1), indicating that in the young needles the photosynthetic apparatus was fully developed. In contrast, young needles also exhibited an increased ratio of Car and xanthophyll cycle pigments to Chl *a*+*b* (Table 1). The young needles absorbed about 10 % less of the incident photons than mature needles (Table 1).

Table 1. Photosynthetic pigments and fractional photon absorbance in young and mature needles of *Cupressus sempervirens* L. (mean±S.E.; n = 5).

	Young needles	Mature needles
Chl *a*+*b* [μmol m^{-2}]	212±10	398±18
Chl *a/b*	3.7±0.3	3.6±0.3
Car/Chl [mmol mol^{-1}]	620±32	465±23
(V+A+Z)/Chl [mol mol^{-1}]	138.0± 6.5	63.0±3.1
Absorbance	0.721±0.002	0.800±0.005

Changes in Chl fluorescence: In order to compare the susceptibility to photoinhibition between young and mature needles, needle samples were subjected to HI in a controlled-environment chamber, followed by a recovery incubated for 60 min in the dark. Young needles were more sensitive to HI than mature needles as indicated by the more pronounced decrease of F_v/F_m ratios in young needles (Fig. 1). Recovery during dark incubation was remarkably fast, so that after 45 min only small differences in F_v/F_m ratios between mature and young needles remained (Fig. 2). The HI-treatment for 60 min led to a decline of F_v/F_m ratio at about 60 and 37 % in young and mature needles, respectively. In contrast, F_0 significantly increased in mature needles (Fig. 2). In the subsequent dark incubation, F_0 decayed with increase in F_v/F_m, and both of them could largely recover after 60 min dark (Fig. 2).

Changes in photosynthetic activities (Fig. 3): The rates of PS2 activities decreased with increasing time of HI exposure in both mature and young needles. After 60 min, the photosynthetic electron transports H_2O→DCBQ and H_2O→SiMo were reduced by about 16 and 56 % in

young and by 32 and 13 % in mature needles, respectively. A significant reduction of PS2 activity was noticed when DCBQ was used as electron acceptor in mature needles but it was marginally inhibited when SiMo was used as electron acceptor (Fig. 3). In contrast, a marked reduction of PS2 activity was noticed using electron acceptor SiMo instead of DCBQ in young needles. A small inhibition of PS1 activity was also evident in both young and mature needles (Fig. 3). In the subsequent dark incubation, the needles reached maximum rate of PS2 activity similar to that observed in non-photoinhibited needles (Fig. 4).

Changes in DCPIP photoreduction: To locate the possible site(s) of inhibition in the PS2 reaction, we followed the DCPIP photoreduction supported by various exogenous electron donors used in thylakoids isolated from 60-min HI-treated mature and young needles. Wydrzynski and Govindjee (1975) found that $MnCl_2$, DPC, NH_2OH, and HQ could donate electrons to the PS2 reaction. Fig. 5 shows the electron transport activity of PS2 in the presence and absence of $MnCl_2$, DPC, and NH_2OH. PS2 activity was reduced to about 67 and 32 % in young and mature needles, respectively, when water served as electron donor. A similar trend was also found using $MnCl_2$ as donor in both needle types. In contrast, we observed a significant restoration of PS2 mediated DCPIP reduction when NH_2OH and DPC were used as electron donors in young needles, while the PS2 activity was not restored using either DPC or NH_2OH in mature needles (Fig. 5).

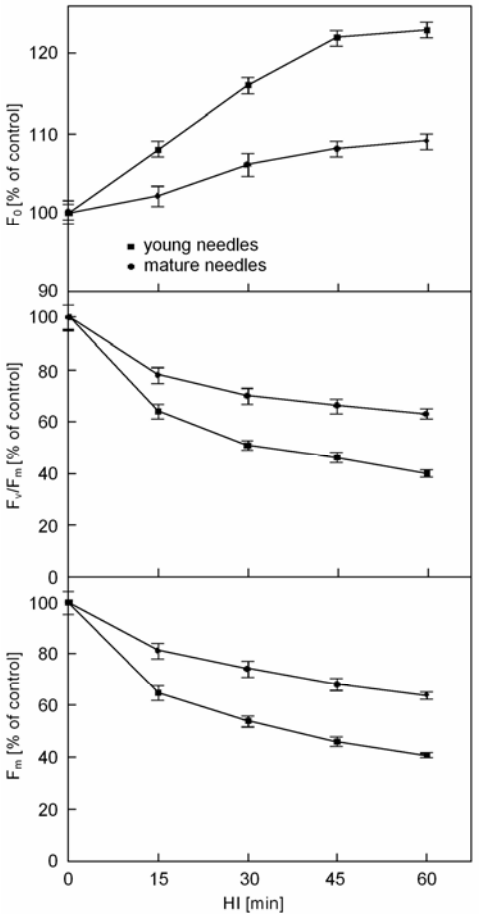

Fig. 1. Changes in the relative fluorescence emitted as minimum fluorescence (F_0), maximum fluorescence (F_m), and the ratio of variable to maximum fluorescence (F_v/F_m) of mature and young needles of cypress at different time intervals of HI-treatments. Values are % of untreated controls. Control values for F_0, F_m, and F_v/F_m were 0.132, 0.680, 0.800 and 0.130, 0.896, 0.816 in young and mature needles, respectively (means±S.E.; n = 5).

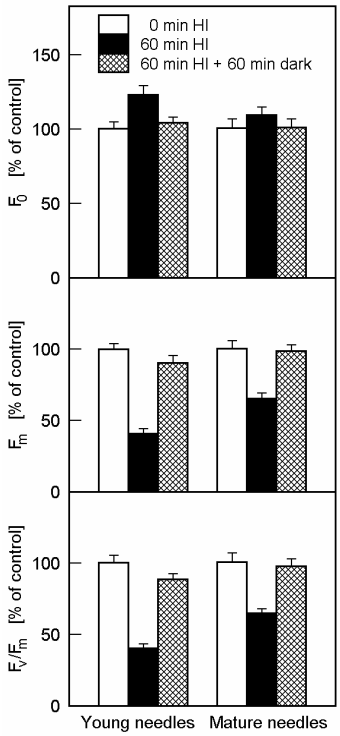

Fig. 2. Photoinhibition and subsequent dark recovery of mature and young needles under controlled conditions as indicated by F_0, F_m, and F_v/F_m. Control values for F_0, F_m, and F_v/F_m were 0.135, 0.780, 0.804 and 0.130, 0.890, 0.832 in young and mature needles, respectively (means±S.E.; n = 5).

Changes in D1 and 33 kDa proteins detected by immunoblotting: Photoinhibition of PS2 induces breakdown of the D1 protein (Andersson and Styring 1991, Prasil *et al.* 1992). In systems without protein biosynthesis this can be seen directly as a loss in content of D1 protein. In the intact plant, the correlation between D1 protein content and activity of PS2 is more complex (Smith *et al.* 1990, Lutz 1992). HI-induced inhibition of PS2 activity in thylakoids of young and mature needles was compared with changes in the relative contents of D1 and 33 kDa proteins as determined by Western blotting followed by quantification by the *Bio-Image* apparatus (Fig. 6). The relative contents of D1 and 33 kDa proteins decreased to 6 or 60 % in young needles and to 30 or 4 % in mature needles irradiated with 60-min HI, respectively.

In the subsequent dark recovery, the needles reached the original contents of D1 in mature and 33 kDa protein in young needles, similar to those observed in non-photoinhibited needles (Fig. 6).

Discussion

The present results pointed out that exposure of young and mature needles to HI produced differential loss of photosynthetic activity and potential efficiency of PS2 (F_v/F_m) where the young needles were more sensitive to HI than the mature needles.

In situ, the young and mature needles of cypress experienced similar exposure to full sunlight. However, the higher Car content per mol Chl *a*+*b* and xanthophyll cycle pigments (Table 1) indicated a strengthened acclimation of the young needles to excess photons (Thiele *et al.* 1997). Young needles are similarly or even more exposed to sun than mature needles and should not be less acclimated to HI. The characteristics of pigment composition, photon absorption and photosynthetic performance of the cypress needles may at least in part explain the differences in susceptibility to photoinhibition: (*a*) Young needles contained around 50 % less Chl per unit leaf area than the mature ones, but photon absorption was only slightly lower in the young needles. The Chl *a/b* ratio, however, was the same in the two needle types, indicating similarly developed antenna systems. (*b*) Young needles exhibited much lower capacities of photosynthetic O_2 evolution, which roughly corresponded to lower Chl content. Due to these two factors, the same irradiation would result in a much higher fraction of excess photons and higher average Chl excitation in the young needles.

The decline in F_v/F_m (used here as a convenient measure of photoinhibition) indicates a reduction in potential PS2 efficiency. In many studies, a close correlation of the F_v/F_m ratio with the quantum yield of photo-

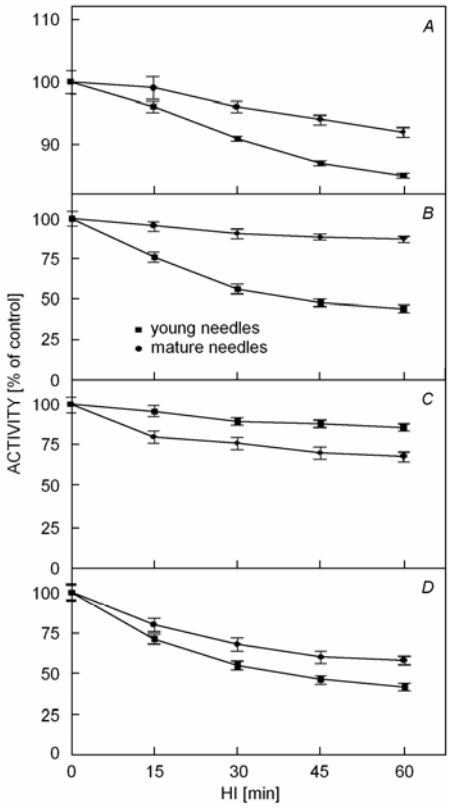

Fig. 3. Changes in the (*A*) $DCPIPH_2 \rightarrow PS1 \rightarrow MV$, (*B*) $H_2O \rightarrow PS2 \rightarrow DCBQ$, (*C*) $H_2O \rightarrow PS2 \rightarrow SiMo$, and (*D*) whole chain $H_2O \rightarrow PS2 \rightarrow PS1 \rightarrow MV$ electron transport activities in thylakoids isolated from HI-treated young and mature needles of cypress at different time intervals. The 100 % values were [mmol(O_2) kg^{-1}(Chl) s^{-1}]: $DCPIPH_2 \rightarrow MV$ 380, 390; $H_2O \rightarrow$ SiMo 75,100; $H_2O \rightarrow DCBQ$ 140, 160; $H_2O \rightarrow MV$ 90, 130 for thylakoids isolated from young and mature needles, respectively (means±S.E.; n = 5).

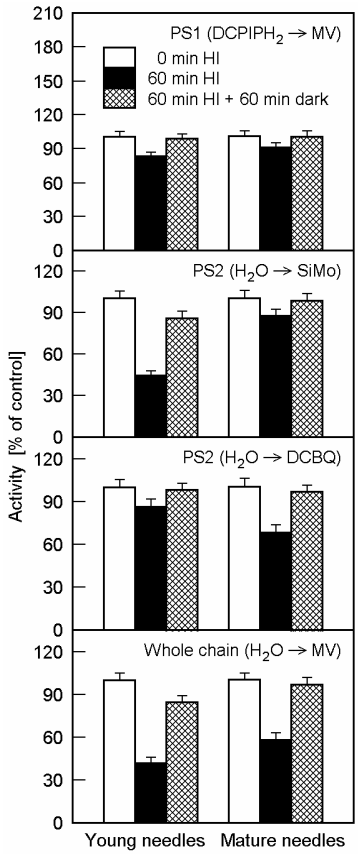

Fig. 4. Photoinhibition and subsequent dark recovery of young and mature needles under controlled conditions as indicated by PS2, PS1, and whole chain electron transport activities at different time intervals. The 100 % values are [mmol(O_2) kg^{-1}(Chl) s^{-1}]: $DCPIPH_2 \rightarrow MV$ 380, 390; $H_2O \rightarrow DCBQ$ 140, 160; $H_2O \rightarrow SiMo$ 70, 100; $H_2O \rightarrow MV$ 90, 130 for thylakoids isolated from young and mature needles, respectively (means±S.E.; n = 5).

synthetic O_2 evolution or CO_2 assimilation under irradiance-limiting conditions has been reported (Krause and Weis 1991, Mulkey and Pearcy 1992, Krause *et al.* 1995). The reduction of F_v/F_m in HI-treated young and mature needles was mainly caused by a decline of F_m and increase of F_0, respectively. An increase of F_0 may be induced by the inactivation of PS2 RCs (Melis 1985, Critchley and Russell 1994, Yamane *et al.* 1997). Our experimental results from mature needles are in accordance with this idea. When F_0 increased in mature needles under HI, some PS2 RCs lost their photochemical activity as indicated by a marked decline in the photochemical efficiency of PS2 (F_v/F_m). Bolhàr-Nordenkampf *et al.* (1991) observed relatively low F_v/F_m ratios; even small changes of F_0 or F_m would result in considerable changes in the F_v/F_m ratio. In the subsequent dark incubation, both young and mature needles reached maximum PS2 photochemistry efficiencies similar to those observed in non-photoinhibited needles. The rate of recovery agrees with other reports on photoinhibition in higher plants (Ogren *et al.* 1984, Öquist *et al.* 1992, Krause *et al.* 1995).

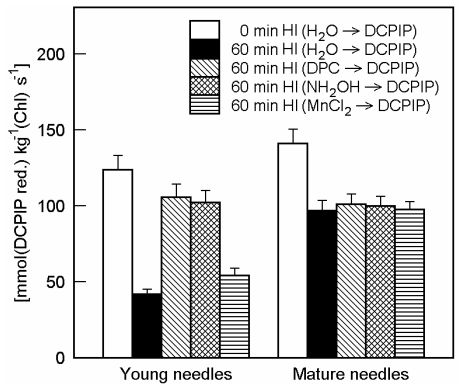

Fig. 5. Effects of various exogenous electron donors on photosystem 2 activity (H_2O→DCPIP) in thylakoids isolated from 60-min HI-treated young and mature needles of cypress. The 100 % values are [mmol(DCPIP red.) kg^{-1}(Chl) s^{-1}]: H_2O→DCPIP 140, 175; DPC→DCPIP 150, 179; NH_2OH→DCPIP 150, 176; $MnCl_2$→DCPIP 130, 175 for thylakoids isolated from young and mature needles, respectively (means±S.E.; n = 5).

As shown by the analysis of electron transport activities in thylakoids isolated from HI-treated mature needles, oxygen evolution was more inhibited when the electron acceptor used was DCBQ than SiMo. This is mainly due to HI-induced changes on the reducing side of PS2 and is due to photoinhibition. The present Chl fluorescence study, where F_0 was markedly increased, supports it. In contrast, in thylakoids isolated from young needles the rate of PS2 activity observed with SiMo was lower than the one observed with DCBQ. This is due to donor side being more impaired than the acceptor side of PS2 because of marked reduction of F_v in young needles. This is characteristic for inhibition of donor side of PS2. If the acceptor side of PS2 is photoinhibited, the F_0 level is significantly increased (Allakhverdiev *et al.* 1987, Šetlík *et al.* 1990). Somersalo and Krause (1990) and Schnettger *et al.* (1994) showed a relationship between F_v/F_m and PS2 electron transport activity in thylakoids isolated from photoinhibited needles.

To locate the possible site of inhibition in the PS2 reaction, we followed the DCPIP photoreduction supported by various exogenous electron donors used in isolated thylakoids from HI-treated mature and young needles. Among the artificial electron donors tested, DPC and NH_2OH donate electrons directly to the RC of PS2 (Wydrzynski and Govindjee 1975). Addition of DPC and NH_2OH markedly restored the loss of PS2 activity in HI-treated young needles. This indicates that the water-oxidizing system of cypress young needles is sensitive to HI. In contrast, DPC or NH_2OH did not restore the loss of PS2 activity in mature needles, indicating that HI does not induce changes on the acceptor side of PS2 of mature needles (Eckert *et al.* 1991, Asada *et al*. 1992, Aro *et al.* 1993, Hong and Xu 1999). Similarly, Schiefthaler *et al.* (1999) observed in field grown *Schefflera arboricola* needles adaptation to different irradiances.

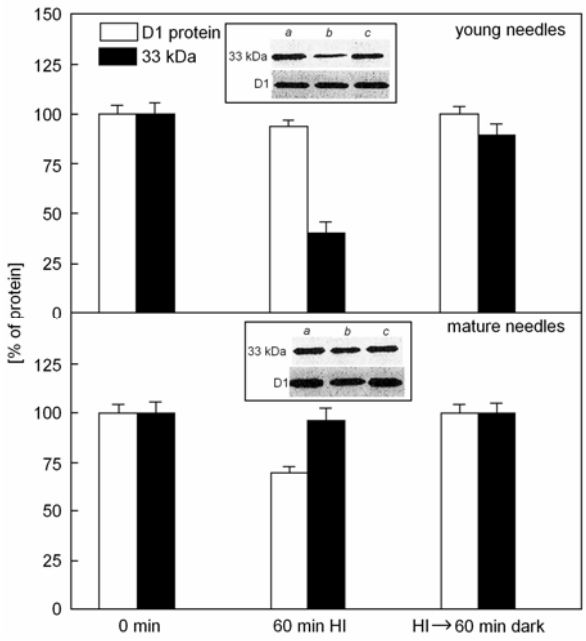

Fig. 6. Relative contents of D1 and 33 kDa proteins of thylakoids isolated from HI-treated and subsequently darkened young and mature needles of cypress at different time intervals. *Lanes*: *a*, 0 min; *b*, 30 min HI; *c*, 60 min HI; *d*, 30 min dark; *e*, 60 min dark.

The loss of PS2 activity could only partially be ascribed to the inhibition of PS2 function, since the 60-min HI-exposure reduced F_v/F_m by about 60 and 37 % in mature needles. We therefore assume that it was mainly due to loss of PS2 centres on a Chl basis. This is supported by the immunological determination of the PS2 RC protein D1 and of the 33 kDa protein of the water-splitting

complex. Photoinhibition is often taken as a result of marked loss of D1 protein in mature needles, because it occurs only when the rate of damage to D1 protein exceeds the rate of its repair (Kyle *et al.* 1984, Barber 1995, Carpentier 1995). Moreover, the fluorescence parameter F_v/F_m may be a good measure of photoinhibition, and the decrease in F_v/F_m under photoinhibitory conditions is often attributed to the loss of D1 protein. Furthermore, after HI-treatment, inactive PS2 RCs accumulate in mature needles or in other needles (Anderson and Aro 1994).

However, as shown by D1 protein quantification, even strong photoinhibition of young needles does probably not induce the loss of the D1 protein in the PS2 RC. In contrast, in mature needles a substantial D1 degradation appeared to be coincident with photoinhibition. This confirms the hypothesis that the D1 protein is not susceptible to photoinactivation in young needles (Thiele *et al.* 1996). We found no correlation between photoinhibition of photosynthesis and loss of the D1 protein when comparing HI-treated young needles. Previous studies on the relationship between photoinhibition and loss of the D1 protein showed either good (Ohad *et al.* 1985) or poor (Hundal *et al.* 1990) correlation. Studies of photoinhibition at low temperatures have also shown that photoinhibition does not correlate with D1 protein degradation *in vivo* (Gong and Nilsen 1989) or *in vitro* (Chow *et al.* 1989). The extrinsic protein of 33 kDa associated with the lumen surface of the thylakoid membranes is required for optimal functioning of the oxygen evolving machinery (Murata *et al.* 1984, Millner *et al.* 1987, Enami *et al.* 1994). Our immunological results indicate that the significant loss of 33-kDa protein could be one of the reasons for significant loss of O_2 evolution capacity in young needles.

We suggest that the high degree of photoinhibition in the young needles indicated by a strong decrease in the F_v/F_m ratio probably reflects a dynamic regulator response of the photosynthetic system to excess of absorbed photon energy. The observed photoinhibition is possibly associated with some loss of productivity but it might protect photosynthetic pigments and electron transport apparatus from severe destruction. The fast recovery probably does not require D1 protein synthesis and may be in relation to xanthophyll cycle activity, which is high in the young needles. Our results also suggest that photoninactivation of PS2 is not correlated at all with the net loss of D1, and photoinhibition represents the formation of inactive centres (Krause 1988, Smith *et al.* 1990, Flexas *et al.* 2001). In addition, we conclude that HI induces changes not only on the acceptor side of PS2 (mature needles) but also on the donor side of PS2 (young needles). Depending on leaf age, HI acclimation probably results in differing degree of PS2 inhibition.

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