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# Photoinhibition of photosynthesis in sun and shade grown leaves of grapevine (*Vitis vinifera* L.)

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## Abstract

The degree of photoinhibition of sun and shade grown leaves of grapevine was determined by means of the ratio of variable to maximum chlorophyll (Chl) fluorescence ($F_v/F_m$) and electron transport measurements. The potential efficiency of photosystem 2 (PS2), $F_v/F_m$, markedly declined under high irradiance (HI) in shade leaves with less than 10 % of $F_0$ level. In contrast, $F_v/F_m$ ratio declined with about 20 % increase of $F_0$ level in sun leaves. In isolated thylakoids, the rate of whole chain and PS2 activity in HI shade and sun leaves was decreased by about 60 and 40 %, respectively. A smaller inhibition of photosystem 1 (PS1) activity was also observed in both leaf types. In the subsequent dark incubation, fast recovery was observed in both leaf types that reached maximum PS2 efficiencies similar to non-photoinhibited control leaves. The artificial exogenous electron donors DPC, $NH_2OH$, and $Mn^{2+}$ failed to restore the HI-induced loss of PS2 activity in sun leaves, while DPC and $NH_2OH$ were significantly restored in shade leaves. Hence HI in shade leaves inactivates on the donor side of PS2 whereas it does at the acceptor side in sun leaves, respectively. Quantification of the PS2 reaction centre protein D1 and the 33 kDa protein of water splitting complex following HI-treatment of leaves showed pronounced differences between shade and sun leaves. The marked loss of PS2 activity in HI leaves was due to the marked loss of D1 protein of the PS2 reaction centre protein and the 33 kDa protein of the water splitting complex in sun and shade leaves, respectively.

*Additional keywords*: chlorophyll fluorescence; donor side; D1 protein; electron transport; photoinhibition; photosystems 1 and 2.

## Introduction

Diurnal and seasonal changes of irradiance in the field range from near darkness to levels well in excess of what is required for maximum photosynthesis. Excess radiant energy has the potential to damage the photosynthetic apparatus and plants have evolved strategies to minimise its detrimental effects. Photoinhibition is most commonly equated with photodamage, a long-term depression of quantum efficiency due to damage to the photosynthetic apparatus as a result of excess photosynthetic photon flux density, PPFD (Walters and Horton 1993). Chronic photoinhibition may be considered as a depression of photosynthetic efficiency from which the plant does not recover after 3–4 d in shade (Greer and Laing 1992). Although photoinhibition has been extensively investigated under controlled conditions, there is still some doubt whether it is a significant factor for plants growing in the field. Ögren and Rosenqvist (1992) argue that moderate photoinhibition is common in field-grown plants. Several investigators view photoinhibition of photosynthesis as a process of stress-induced damage to photosystem 2 (PS2). This is based on the fact that, as a consquence of photoinhibition, the D1 protein of PS2 reaction centre becomes degraded (Prášil *et al*. 1992, Rintamaki *et al*. 1995). But some recent reports suggest that photoinhibition, first of all, results from the formation of photochemically inactive PS2 centres, which convert the excitation energy into heat. This down regulation of PS2 and thermal dissipation is considered as

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*Abbreviations:* 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; SiMo – silicomolybdate.
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a protective mechanism against HI stress (Aro *et al*. 1993, Gilmore and Björkman 1994). The photoinactivation and impairment of electron transport occur 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).

Shade plants are more prone to photoinhibition of photosynthesis than are sun plants (Powles 1984, Anderson and Osmond 1987). The higher sensitivity of shade plants to photoinhibition is generally ascribed to factors such as HI-absorbing chlorophyll (Chl) antenna for PS2 and lower rates of photon saturated photosynthesis. In the cyanobacterium *Anacystis nidulans*, low irradiance grown cells had a lower capacity to recover from photoinhibition than had HI-grown cells (Samuelsson *et al*. 1987, Lönneborg *et al*. 1988). This difference in ability to recover from photoinhibition was attributed to different rates of the repair cycle of PS2 with a higher turnover of PS2 in HI-grown cells conferring significant protection against photoinhibition.

The effect of photoinhibition, alone or interacting with other stresses, on the long-term consequences of carbon balance or productivity, is little known. 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. In freshwater phytoplankton, for instance, recovery of photosynthesis occurred during 4–20 h, depending on the extent of the inhibition (Belay 1981). Similarly, the recovery of the photon yield of leaves of *Phaseolus vulgaris* took between 4 and 8 h (Powles *et al*. 1983).

The D1 reaction centre protein of PS2 is a target of HI-induced damage to the PS2 complex; turnover of the D1 protein is accelerated by increasing irradiance (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. Low irradiance or shade-grown plants are more susceptible to photoinhibition than HI or sun grown species (Powles 1984, Aro *et al*. 1993, Öquist *et al*. 1994). This higher susceptibility is accompanied by slow degradation of D1 protein (Tyystjärvi *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-translation processing and modification of the protein, and ligation of the electron-transfer components (Aro *et al*. 1993). Thiele *et al*. (1996) suggested that, like in cold-acclimated spinach, the D1 protein is stabilised in young and mature canopy leaves and in D1 inactivation, and that turnover takes little part in photoinhibition and recovery.

The object of our work was to compare the susceptibility to photoinhibition and the process of recovery in shade grown and sun grown leaves of *Vitis vinifera*. The effect of photoinhibition was analysed by fluorescence with respect to photosynthetic oxygen evolution and potential PS2 function. The amounts of D1 and 33 kDa proteins were also analysed 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 analysed by establishing rates of recovery from photoinhibition.

## Materials and methods

**Plants**: 13-year-old grapevine (*Vitis vinifera* L.) plants were grown under field conditions in San Michele all' Adige, Italy (46°12'N, 11°08'E). Vines were trained to a permanent cordon at 1.80×0.80 m spacing, with upright growing shoots. Plants grown at full sunlight (maximum 2 000 μmol $m^{-2}$ $s^{-1}$) were designated as sun leaves, while plants grown at 40 % of sunlight (maximum 800 μmol $m^{-2}$ $s^{-1}$) were designated as shade leaves. Reduced irradiances were obtained by screening full sunlight through appropriate nylon green meshes (1 m above the canopy). The fully expanded leaves (40-d-old) were taken as experimental samples. Leaves were sampled early in the morning before they had experienced direct sunlight.

**Photoinhibition and recovery under controlled conditions**: Detached leaves were placed 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 ºC and relative humidity was 66±5 %. The PPFD was measured with a quantum sensor (*LI-Cor*, Lincoln, NE, USA). Leaf temperatures, recorded with thermocouple attached to the lower surface, were between 27 and 29 ºC. Discs of 1.6 $cm^2$ area were punched from the leaf blades after specified times of HI exposure and placed on moist filter paper in petri dishes (temperature 25–27 ºC). The leaf discs were darkened for 10 min before the degree of photoinhibition was determined by fluorescence measurement. For recovery from photoinhibition the leaf discs were kept in complete dark for 60 min.

**Modulated Chl fluorescence in leaves** was measured on leaf discs using a *PAM 2000* fluorometer (*H. Walz*, Effeltrich, FRG). Before the measurements, the leaves were dark adapted for 10 min. $F_0$ was measured by switching on the modulated radiation of 0.6 kHz; PPFD was less than 0.1 μmol $m^{-2}$ $s^{-1}$ at the leaf surface. $F_m$ was measured at 20 kHz with a 1 s pulse of 6 000 μmol $m^{-2}$ $s^{-1}$ of "white light".

**Activities of electron transport**: Thylakoid membranes were isolated at 4 ºC, from each sample six to eight disks, as described by Russell *et al*. (1995). Leaf disks were homogenised in an ice cold grinding medium containing 25 mM Tris-HCl, pH (7.8), 10 mM NaCl, 5 mM $MgCl_2$, and 330 mM sucrose. The homogenate was filtered rapidly through four layers of *Miracloth* and thylakoids were collected by centrifugation at 9 000×*g* for 5 min.

Whole chain electron transport ($H_2O \rightarrow MV$) and partial reactions of photosynthetic electron transport mediated by PS2 ($H_2O \rightarrow DCBQ$; $H_2O \rightarrow SiMo$) and PS1 ($DCPIPH_2 \rightarrow MV$) 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** 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**: The relative contents of certain thylakoid proteins per Chl unit were determined immunologically by Western blotting. Thylakoids were solubilised 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 electroblotting for 3 h at 0.4 A after saturation with 10 % milk powder in 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 second 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). The antibody against 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 Chl fluorescence**: In order to compare the susceptibility to photoinhibition between sun and shade leaves, leaf samples were subjected to HI of variable duration (Fig. 1) in a controlled-environment chamber and followed by 60 min dark incubation for recovery (Fig. 2). Fig. 1 illustrates that shade leaves responded more sensitively to HI than sun leaves, as indicated by the more pronounced decrease in $F_v/F_m$ ratios of shade leaves. The levels of $F_0$, $F_m$, and $F_v/F_m$ were recovered fast in first 30-min dark incubation and after only small differences in both sun and shade leaves (Fig. 2). The HI treatment for 60 min lead to a decline of about 38 or 61 % in $F_v/F_m$ and elevation of about 19 or 5 % in $F_0$ in sun and shade leaves, respectively. In the subsequent dark incubation for 60 min, the level of $F_0$ recovered completely in both leaf types while the level of $F_v$ and $F_v/F_m$ ratio did not recover completely (Fig. 2).

**Changes in photosynthetic activities**: Photosynthetic electron transport activities were measured in thylakoids isolated from HI-treated sun and shade grown leaves (Fig. 3). The rate of PS2 activity was decreased with increase in the time of HI in both sun and shade leaves. After 60 min, photosynthetic electron transports from $H_2O \rightarrow DCBQ$ and $H_2O \rightarrow SiMo$ were reduced by about 38 or 9 % in sun and 10 or 56 % in shade leaves, respectively. A significant reduction of PS2 activity was noticed when DCBQ was used as electron acceptor in sun leaves but it was marginally inhibited when SiMo was used

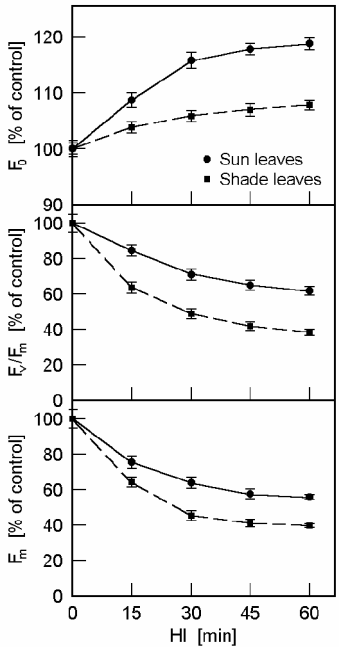


Fig. 1. Changes in the relative fluorescence emitted as minimal fluorescence ($F_0$), maximum fluorescence ($F_m$), and the ratio of variable to maximum fluorescence ($F_v/F_m$) of sun and shade grown leaves of *Vitis vinifera* at different duration of high irradiance (HI). Data are given in % of untreated controls. Control values for $F_0$, $F_m$, and $F_v/F_m$ were 2.6, 11.2, 0.811 and 2.6, 10.2, 0.798 in sun and shade grown leaves, respectively (mean ± S.E.; $n = 5$).

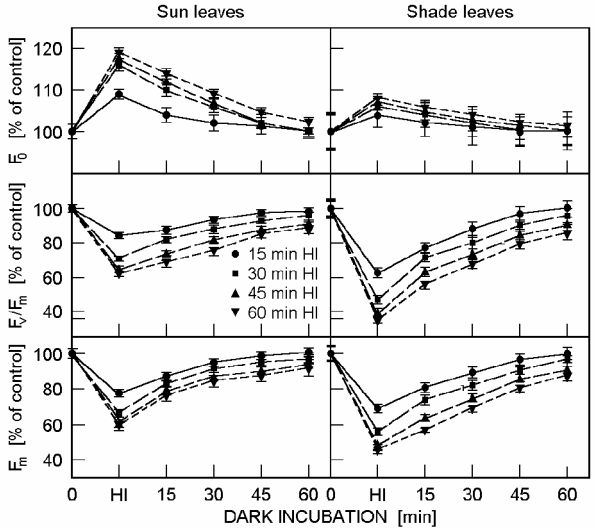


Fig. 2. Photoinhibition and subsequent dark recovery of sun and shade grown leaves under controlled conditions as indicated by $F_0$, $F_m$, and $F_v/F_m$. Control values for $F_0$, $F_m$, and $F_v/F_m$ were 2.6, 11.2, 0.811 and 2.6, 10.2, 0.798 in sun and shade leaves, respectively (mean ± S.E.; $n$ = 5).

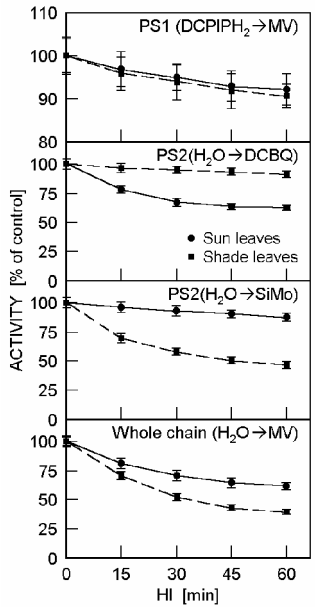


Fig. 3. Changes in the rates of whole chain ($H_2O$→MV), PS2 ($H_2O$→DCBQ; $H_2O$→SiMo), and PS1 ($DCPIPH_2$→MV) electron transport activities in thylakoids isolated from high irradiance (HI) treated sun and shade leaves of *Vitis vinifera* at different time intervals. The 100 % values are [mmol($O_2$) $kg^{-1}$(Chl) $s^{-1}$]: $DCPIPH_2$→MV 396, 309; $H_2O$→DCBQ 168, 141; $H_2O$→SiMo 102, 49; $H_2O$→MV 139, 52 for thylakoids isolated from sun and shade leaves, respectively (mean ± S.E.; $n$ = 5).

(Fig. 3). In contrast, a marked reduction of PS2 activity was found when SiMo was used instead of DCBQ in shade leaves. A smaller inhibition of PS1 activity was also observed in both sun and shade leaves (Fig. 3). In the subsequent dark incubation, the leaves reached maximum PS2 activity similar to activity observed in non-photoinhibited leaves (Fig. 4).

**Changes in DCPIP photoreduction measurements**: 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 sun and shade leaves. Wydrzynski and Govindjee (1975) have shown that $MnCl_2$, DPC, $NH_2OH$, and HQ donate electrons in the PS2 reaction. Fig. 5 shows the electron transport activity of PS2 in the presence and absence of three of the above compounds. The PS2 activity was reduced to about 35 % in sun leaves and 60 % in shade leaves, when water served as electron donor. A similar trend was also found using $MnCl_2$ as donor in both leaf types. In contrast, a significant restoration of PS2 mediated DCPIP reduction was observed when $NH_2OH$ and DPC were used as electron donors in shade leaves, while the PS2 activity was not restored using either DPC or $NH_2OH$ in sun leaves (Fig. 5).

**Changes in D1 and 33 kDa proteins studied by immunoblot**: Photoinhibition of PS2 induces breakdown of the D1 protein (Andersson and Styring 1991, Prášil *et al.*

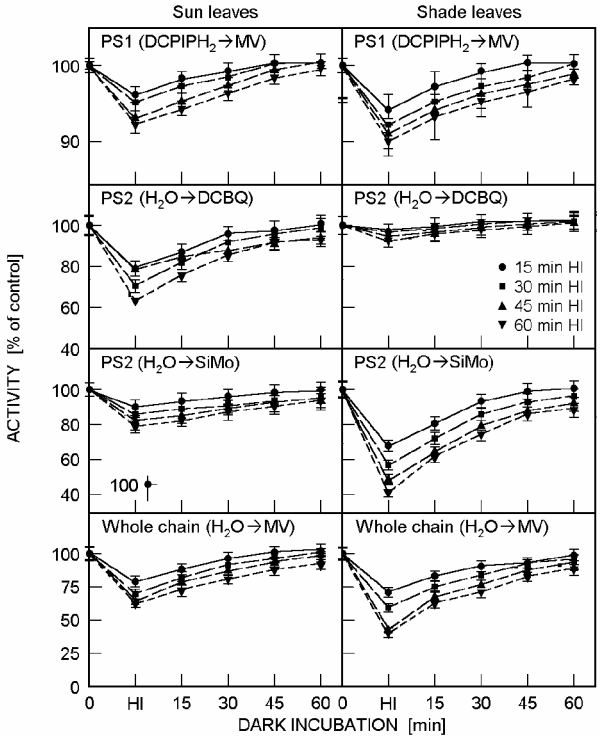


Fig. 4. Photoinhibition and subsequent dark recovery of sun and shade grown leaves under controlled conditions as indicated by PS2, PS1, and whole chain electron transport activities at different time intervals. The 100 % values are [mmol($O_2$) $kg^{-1}$(Chl) $s^{-1}$]: $DCPIPH_2$→MV 396, 309; $H_2O$→DCBQ 168, 141; $H_2O$→SiMo 102, 49; $H_2O$→MV 139, 52 for thylakoids isolated from sun and shade leaves, respectively (mean ± S.E.; $n$ = 5).

1992). In systems without protein biosynthesis this can be seen directly as a loss in D1 protein content. In intact plant the correlation between D1 protein content and activity of PS2 is more complex (Smith *et al*. 1990, Lutz *et al*. 1992). Photoinhibition induced inhibition of PS2 activity in thylakoids of sun and shade grown leaves was compared with changes in the relative contents of D1 and 33 kDa proteins as determined by Western blotting (Fig. 6) followed by quantification by the *Bio-Image* apparatus (Fig. 6). The relative contents of D1 and 33 kDa proteins decreased to 58 or 3 % and 2 or 68 % in 60 min HI-treated sun and shade leaves, respectively. In the subsequent 60 min of dark incubation, the leaves reached the original contents of D1 in sun leaves and of 33 kDa protein in shade leaves, similar to observation in non-photoinhibited leaves (Fig. 6).

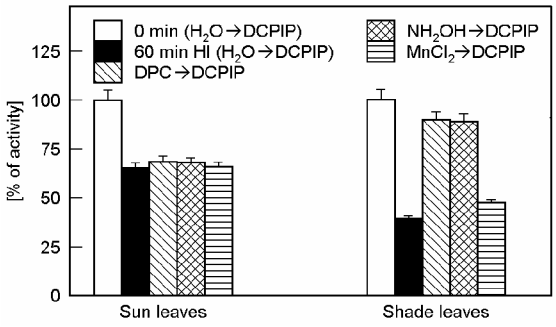


Fig. 5. Effect of various exogenous electron donors on photosystem 2 (PS2) activity ($H_2O$→DCPIP) in thylakoids isolated from 60 min high irradiance (HI) treated sun and shade grown leaves of *Vitis vinifera*. The 100 % values are [mmol(DCPIP red.) $kg^{-1}$(Chl) $s^{-1}$]: $H_2O$→DCPIP 176, 78; DPC→DCPIP 179, 158; $NH_2OH$→DCPIP 180, 154; $MnCl_2$→DCPIP 77, 87 for thylakoids isolated from sun and shade grown leaves, respectively (mean ± S.E.; $n$ = 5).

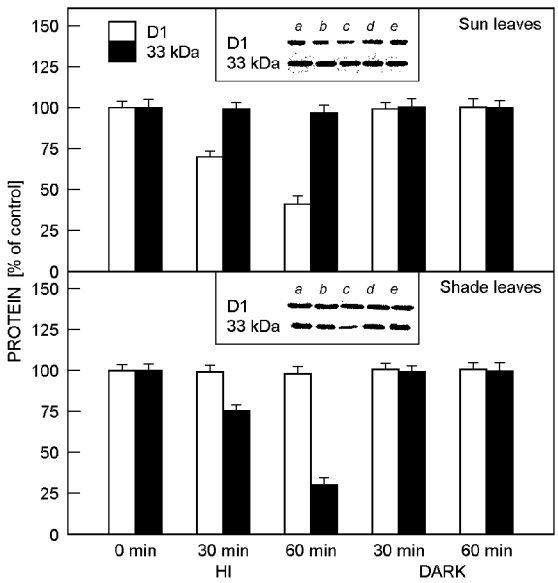


Fig. 6. Relative contents of D1 and 33 kDa proteins of thylakoids isolated from high irradiance (HI) treatment followed by dark incubation in sun and shade grown leaves of *Vitis vinifera* at different time intervals. Lane *a*, 0 min; lane *b*, 30 min HI; lane *c*, 60 min HI; lane *d*, 30 min dark; lane *e*, 60 min dark. Each lane was loaded with equal amount of Chl (5 μg). *Histogram*: *Bio-Image* densitometric evaluation. *Inset*: Western-blot.

In Fig. 7, relative D1 protein contents and $F_v/F_m$ ratios are compared after photoinhibitory treatments of sun and shade leaves. In the shade leaves, no significant D1 degradation could be attributed to the action of photoinhibitory radiation, even when $F_v/F_m$ decreased to 58–61 % of the controls. The sun leaves showed a strong decrease in D1 protein (58 %) content together with the decline of $F_v/F_m$ ratio to about 38 % (Fig. 7).

## Discussion

The present results indicate that exposure of shade and sun grown leaves to HI produces differential loss of photosynthetic activity and potential efficiency of PS2 ($F_v/F_m$) where the shade leaves are more sensitive to HI than the sun leaves.

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 photosynthetic $O_2$ evolution or $CO_2$ assimilation under PPFD-limiting conditions has been reported (Krause and Weis 1991, Mulkey and Pearcy 1992). The reduction of $F_v/F_m$ in HI-treated sun leaves was mainly caused by marked increase of $F_0$. An increase of $F_0$ may be induced by the inactivation of part of PS2 reaction centres (Melis 1985, Critchley and Russell 1994, Yamane *et al*. 1997). Our experimental results from sun grown leaves are in accordance with this idea. The decrease of $F_v/F_m$ in photoinhibited shade leaves is mainly due to the significant decrease of $F_m$ and marginal increase of $F_0$. Bolhar-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 shade grown and sun grown leaves reached maximum PS2 photochemistry efficiencies similar to those observed in non-photoinhibited leaves. The rate of recovery agrees with other reports on photoinhibition in higher plants (Ögren *et al*. 1984, Öquist *et al*. 1992).

As shown by the analysis of electron transport activities in thylakoids isolated from HI-treated sun leaves, the oxygen evolution was inhibited markedly when the electron acceptor used was DCBQ, but not SiMo. This is mainly due to HI-induced changes on the reducing side of PS2, *i.e*. photoinhibition. Chl fluorescence studies where $F_0$ was markedly increased support it (Asada *et al*. 1992,

Endo *et al*. 1998). In contrast, in thylakoids isolated from shade grown leaves the PS2 activity observed with SiMo was lower than that observed with DCBQ. This is due to donor side being more impaired than the acceptor side of PS2. The extent of variable fluorescence ($F_v$) was reduced markedly with slight increase of $F_0$ level. This is characteristic for inhibition of donor side of PS2 (Allakhverdiev *et al*. 1987, Šetlík *et al*. 1990). A relation between $F_v/F_m$ and PS2 electron transport activity in thylakoids isolated from photoinhibited leaves has also been shown (Somersalo and Krause 1990, Schnettger *et al*. 1994). In the subsequent dark incubation, both shade and sun leaves reached maximum PS2 activity comparable to non-photoinhibited leaves.

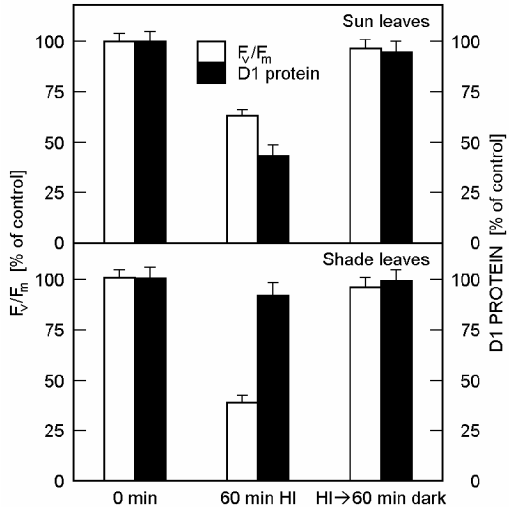


Fig. 7. Quantification of D1 protein and degree of photoinhibition in *Vitis vinifera* leaves under controlled conditions. $F_v/F_m$ ratios were determined as a measure of photoinhibition. Data are given in % of untreated controls (mean ± S.E.; $n = 5$).

To locate the possible site of inhibition in the PS2 reaction, we followed the DCPIP photoreduction supported by various exogenous electron donors in thylakoids isolated from 60 min HI of both sun and shade leaves. Among the artificial electron donors tested, DPC and $NH_2OH$ donate electrons directly to the reaction centre of PS2 (Wydrzynski and Govindjee 1975). Addition of DPC and $NH_2OH$ markedly restored the HI-induced loss of PS2 activity in shade leaves. This is because the water-oxidizing system is sensitive to HI in grapevine shade leaves. In contrast, using DPC or $NH_2OH$ in sun leaves did not restore the loss of PS2 activity. Hence HI induces changes on the acceptor side of PS2 in sun-grown leaves (Asada *et al*. 1992, Aro *et al*. 1993, Hong and Xu 1999). Schiefthaler *et al*. (1999) found similar results for field grown *Schefflera arboricola* leaves adapted to various PPFD.

The loss of PS2 activity could only partially be ascribed to functional inhibition of PS2 since $F_v/F_m$ was reduced by about 61 and 38 % in 60-min HI-irradiated shade and sun leaves, respectively. We therefore assume that it was mainly due to loss of PS2 reaction centres and water splitting complex protein. This was confirmed by the immunological determination of the PS2 reaction centre protein of D1 and the 33 kDa protein of the water splitting complex. It is often thought that photoinhibition is a result of marked loss of D1 protein in sun grown leaves. So it occurs only when the rate of damage to D1 protein exceeds the rate of its repair (Barber 1995, Carpentier 1997). Moreover, the fluorescence parameter $F_v/F_m$ is considered to be a good measure of photoinhibition, and a decrease in $F_v/F_m$ under photoinhibitory conditions is often attributed to the loss of D1 protein. Furthermore, after HI-treatment, inactive PS2 reaction centres were accumulated in sun leaves or in other leaves (Anderson and Aro 1994).

However, as shown by D1 protein quantification, even strong photoinhibition of shade leaves does not seem to be related to loss of the D1 protein in the PS2 reaction centre (Chow *et al*. 1989, Gong and Nilsen 1989). Similarly, the shade plant *Tradescantia* showed no D1 protein degradation after photoinhibition (Öquist *et al*. 1992). These findings are consistent with our hypothesis of a slower degradation of photoinhibited PS2 reaction centres in shade plants, thereby allowing a significant amount of photoinhibited PS2 reaction centres to remain physically intact under prevailing HI. In contrast, in sun leaves (Fig. 7) a substantial D1 degradation was coincident with photoinhibition (Thiele *et al*. 1996). We found no correlation between photoinhibition of photosynthesis and loss of the D1 protein when HI-treated shade leaves were compared. Previous studies on the relationship between photoinhibition and loss of the D1 protein show either good (Ohad *et al*. 1985) or poor (Hundal *et al*. 1990) correlation. In the subsequent dark incubation, a high amount of D1 protein recovered in photoinhibited sun leaves. We suggest that this is a 'sun-plant defence strategy'. A high reliance on the PS2 repair cycle involving *de-novo* D1 protein synthesis to counteract photoinhibition is exemplified also in HI-acclimated cyanobacteria (Samuelsson *et al*. 1987). However, the general significance of the PS2 repair cycle for the protection from photoinhibition even in HI-acclimated plants must be viewed cautiously. Efficient recovery from photoinhibition only occurs under low irradiance (Samuelsson *et al*. 1987, Greer and Laing 1992). Possibly, the PS2 repair cycle is more important for protection against photoinhibition in rapidly turning-over cyanobacteria (Samuelsson *et al*. 1987) than in mature leaves of higher plants.

The extrinsic protein of 33 kDa associated with the lumenal 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 the 33 kDa protein could be one of the reasons for significant loss of $O_2$ evolution capacity in shade

grown leaves.

Our results suggest that the high degree of photoinhibition in the shade grown leaves indicated by a strong decrease in $F_v/F_m$ 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 might protect photosynthetic pigments and electron transport apparatus from severe destruction. Our results also suggest that photoinactivation 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 concluded that HI not only changes the acceptor side of PS2 (sun leaves) but also the donor side of PS2 (shade leaves). Depending on the leaf type (sun and shade) and irradiance acclimation probably results in differing degree of PS2 inhibition.

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