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Leaf pigments, ribulose-1,5-bisphosphate carboxylase, nitrate reductase and photosynthetic efficiency of grapevine (*Vitis vinifera* L. cv. Pinot noir) grown under different light conditions

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Summary

Changes of leaf pigments, ribulose-1,5-bisphosphate carboxylase (Rubisco), nitrate reductase and photosynthetic efficiency were determined in leaves of *Vitis vinifera* L. cv. Pinot noir plants grown at full sunlight (2000 μmol m^{-2} s^{-1}) and 40 % of sunlight (800 μmol m^{-2} s^{-1}). The contents of chlorophyll and carotenoids per fresh mass were higher in 40 % sunlight than in full sunlight-grown leaves. In contrast, Rubisco activity, *in vivo* nitrate reductase activity (indicator of nitrate utilisation) and soluble proteins were significantly reduced in 40 % sunlight-grown leaves. In isolated thylakoids, a marked inhibition of whole chain (PSI+PSII) and PSII activity were observed in 40 % sunlight-grown leaves. Smaller inhibition of PSI activity was also observed in 40 % sunlight-grown leaves. The artificial exogenous electron donors, DPC and NH_2OH, significantly restored the loss of PSII activity in 40 % sunlight-grown leaves. The same results were obtained when Fv/Fm was evaluated by chlorophyll fluorescence measurements. The marked loss of PSII activity in 40 % sunlight-grown leaves was due to the loss of 47, 33, 28-25 and 23 kDa polypeptides. This conclusion was confirmed by immunological studies showing that the content of the 33 kDa protein of the water-splitting complex was diminished significantly in 40 % sunlight-grown leaves.

K e y w o r d s : chlorophyll fluorescence, electron transport, donor side, nitrate reductase, photosystem.

A b b r e v i a t i o n s : Car = carotenoids, Chl = chlorophyll, DCBQ = 2,6-dichloro-p-benzoquinone, DCPIP = 2,6-dichlorophenol indophenol, DPC = diphenyl carbazide, Fo = minimal fluorescence, Fm = maximum fluorescence, Fv = variable fluorescence, MV = methyl viologen, PS = photosystem; Rubisco = ribulose-1,5-bisphosphate carboxylase, SDS-PAGE = sodium dodecylsulphate - polyacrylamide gel electrophoresis, SiMo = silicomolybdate.

Introduction

To attain light saturation sun leaves generally require a higher photon flux density and have a higher maximum photosynthetic rate and light compensation point than corresponding shade leaves. However, the basis (Charles-Edwards and Ludwig 1975) on which the photosynthetic rate is expressed is important, because in general sun leaves are thicker than shade leaves (Björkman *et al*. 1973, McClenden and McMillen 1982).

Plants growing under high light conditions absorb large amounts of light energy and can sustain high photosynthetic rates. Chloroplasts of sun leaves contain less chlorophyll in their antennae, but relatively more reaction centers and components of the electron transport chain than chloroplasts of shade leaves (Anderson 1986). For plants growing in the shade, light energy is a limiting factor; in order to maximize photon absorption, they produce leaves with larger areas and higher chlorophyll content per chloroplast. Their chloroplasts are characterized by large grana stacks, with most of the chlorophyll in the outer antenna, the light-harvesting complex. Other parts of the photosynthetic apparatus are relatively small (Evans 1988).

Extensive research on sun-to-shade adaptation in leaves has demonstrated that low light-grown plants adapt to light-limited growth conditions by increasing the light harvesting ability and alter chloroplast anatomy by increasing the amount of appressed regions in thylakoid membranes (Anderson 1986). In shade-adapted leaves adjustment of the level of Chl b, light harvesting complex proteins, and additional changes in Rubisco and Q_B protein levels are mostly regulated by changes in gene expression at the transcriptional or post transcriptional level (Senger and Bauer 1987).

Grapevine canopies consist of leaves of different ages, which are subjected to variable light intensities during the growing season (Hunter and Visser 1988). According to Boardman (1977) a leaf's photosynthetic productivity is primarily governed by its position in the plant canopy. Therefore, it would be of interest to determine the changes in chlorophyll concentration of the leaves as well as the relationship, if any, with different photosynthetic activities. This was studied by Hunter and Visser (1988), especially with respect to partial defoliation. In the present paper, we report the effect of different irradiance intensities on changes in leaf pigments, ribulose-1,5-bisphosphate carboxylase, nitrate reductase and photosynthetic efficiency of grapevine (cv. Pinot noir) grown under field conditions.

Material and Methods

P l a n t g r o w t h : Investigations were carried out with 13-year-old grapevines (Vitis vinifera L. cv. Pinot noir, grafted to 3309 C) grown under field conditions in San Michele

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all'Adige, Italy (46 °12' North, 11° 08' East). Vines were trained to a permanent cordon at 1.80 m x 0.8 m spacing, with upright growing shoots and pruned to 10 buds. Shoots were trimmed to 14 leaves when the 17th leaf appeared. Only 5 lateral shoots were selected for each principal shoot they were trimmed to 4 leaves. The plants were classified according to the light intensity at the leaf surface. One group of plants was grown at full sunlight (maximal 2000 μmol m^{-2} s^{-1}, 'full sunlight-grown plants'), while the other was grown at 40 % of sunlight (maximal 800 μmol m^{-2} s^{-1}, '40 % sunlight-grown plants'). Reduced irradiance was obtained by screening full sunlight through appropriate green nylon meshes (1 m above the canopy). Fully expanded basal leaves (40 d old), inserted opposite to the 1st grape, were used as experimental leaves on 8th June. Leaves were sampled early in the morning before they had experienced direct sunlight. From leaf appearance (1st May) to leaf sampling (8th June) the average daily maximum air temperature was 25.6 °C (SD 3.7 °C), the highest air temperature was 33.5 °C (29th May). The average daily minimum temperature was 12.0 °C (SD 2.9 °C), the lowest air temperature was 4.2 °C (4th June). The maximal leaf temperature (29th May) on full sunlight-grown plants was 36 °C and 30 °C on 40 % sunlight-grown plants.

Pigment determination: Chl was extracted with 100 % acetone from leaves frozen in liquid N_2 and stored at -20 °C. Chl and Car were analyzed spectrophotometrically according to LICHTENTHALER (1987).

Chl fluorescence in leaves and thylakoid membranes: Chl fluorescence was measured on leaf discs using a PAM 2000 chlorophyll fluorescence meter (H. Walz, Effeltrich, Germany). Fo was measured by switching on the modulated light to 0.6 kHz; PPFD was less than 0.1 μmol m^{-2} s^{-1} at the leaf surface. Fm was measured at 20 kHz with a 1s pulse of 6000 μmol m^{-2} s^{-1} of white light.

Modulated Chl fluorescence of isolated thylakoid membranes at room temperature was also measured with a PAM 2000. Measurements were performed in 0.7 ml reaction mixture containing 50 mM Tris-HCl (pH 7.5), 2 mM $MgCl_2$, 10 mM NaCl, 100 mM sucrose and 10 μg Chl equivalent thylakoid membranes. The integrated intensity of measuring light (480 nm) was 0.15 μmol m^{-2} s^{-1} with a red actinic light (650 nm) intensity of 100 μmol m^{-2} s^{-1} .

Electron transport measurements: Thylakoid membranes were isolated from the leaves as described by BERTHHOLD *et al*. (1981). Whole chain electron transport (H_2O MV) and partial reactions of photosynthetic electron transport mediated by PSII (H_2O DCBQ; H_2O SiMo) and PSI ($DCPIPH_2$ MV) were measured as described by NEDUNCHEZHIAN *et al*. (1997). Thylakoid membranes were suspended at 10 μg Chl ml^{-1} 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 500 μM DCBQ and 200 μM SiMo.

DCPIP photoreduction measurements: DCPIP reduction was determined as the decrease in absorbance at 590 nm using a Hitachi 557 spectrophotometer. The reaction mixture (3 ml) contained 20 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, 10 mM NaCl, 100 mM sucrose, 100 mM DCPIP and thylakoids equivalent to 20 μg of Chl. Where mentioned, the concentrations of $MnCl_2$, DPC and NH_2OH were 5, 0.5 and 5 mM, respectively.

SDS-PAGE: Thylakoid membranes were separated using the polyacrylamide gel system of LAEMMLI (1970), with the following modifications. Gels consisted of a 12-18 % gradient of polyacrylamide containing 4 M urea. Samples of thylakoid membrane preparations were solubilized at 20 °C for 5 min in 2 % (w/v) SDS and 60 mM DTT and 8 % sucrose using SDS/Chl ratio of 20:1. Electrophoresis was performed at 20 °C with constant current of 5 mA. Gels were stained in methanol/acetic acid/water (4:1:5, v/v/v) containing 0.1 % (w/v) coomassie brilliant blue R and destained in methanol/acetic acid/water (4:1:5, v/v/v). The thylakoid membrane protein was estimated according to LOWRY *et al.* (1951).

Immunological determination of thylakoid membrane proteins: The relative content of certain thylakoid proteins per mg chlorophyll was determined immunologically by western blotting. Thylakoids were solublized 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 above 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 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, Jerusalem, Israel); the antibody against the 33 kDa protein of the water-splitting system was a gift from Dr. BARBATO, Padova, Italy. The densitometry analysis of western blots was performed with a Bio-Image analyser (Millipore Corporation, Michigan, USA).

Determination of soluble proteins: Soluble protein was extracted by grinding leaves (0.3-0.5 g fresh mass) in a mortar with 6 ml of 100 mM Tris-HCl, pH 7.8 containing 15 mM $MgCl_2$, 1 mM EDTA, 10 mM 2-mercaptoethanol, 10 mM PMSF in the presence of liquid nitrogen. Homogenates were fitered through a nylon cloth. After centrifugation at 11,000 *g* for 10 min, the concentration of soluble proteins was determined in the supernatant according to BRADFORD (1976).

Extracts and assay of Rubisco activity: Leaves were cut into small pieces and homogenized in a grinding medium consisting of 50 mM Tris-HCl, pH 7.8, 10 mM $MgCl_2$, 5 mM DTT and 0.25 mM EDTA. The extract was clarified by centrifugation at 10,000 *g* for 10 min. The clear supernatant was decanted slowly and used as Rubisco. Rubisco activity was measured as described by NEDUNCHEZHIAN and KULANDAIVELU (1991).

Nitrate reductase activity: Leaves (100 mg) were suspended in a glass vial containing 5 ml of the assay medium consisting of 100 mM KH_2PO_4-KOH, pH 7.0, 100 mM KNO_3, 1 % (v/v) n-propanol. The vial was sealed and incubated in the dark at room temperature at 27 °C for 60 min. Suitable aliquots of the assay medium were removed for nitrate analysis. The amount of nitrate formed was expressed as μmol NO_2^- formed g^{-1} tissue h^{-1} (JAWORSKI 1971).

Results

Leaf pigments: A fresh mass basis, Chl and Car values of 40 % sunlight-grown leaves were increased by 23 and 28 %, respectively, as compared to full sunlight-grown leaves. The changes in total Chl content could be attributed to the changes in Chl a and Chl b, the Chl a/b ratio being markedly decreased in 40 % sunlight-grown leaves (Tab. 1).

Chl fluorescence in leaves: To obtain information on PSII activity, Fv/Fm, which reflects the potential quantum yield of PSII photochemistry (Krause and Weis 1991), was determined *in vivo* using leaves which had been dark-adapted for 30 min. The effect of 40 % sunlight was prominent on the variable part of fluorescence without changing in Fo. Fm and Fv/Fm were much lower in 40 % sunlight-grown leaves than in full sunlight-grown leaves (Fig. 1). The value of Fv/Fm in full sunlight-grown leaves was 0.797 and the ratio was decreased to about 0.714 in 40 % sunlight-grown leaves.

Photosynthetic electron transport activities: When photosynthetic electron transport was studied using isolated thylakoids from full and 40 % sunlight-grown leaves, the rate of $DCPIPH_2$ MV (PSI) was about 8 % lower in 40 % sunlight-grown leaves as compared to full sunlight-grown leaves (Fig. 2). The PSII activities measured as H_2O DCBQ and H_2O SiMo were about 16 % and 52 % lower in 40 % sunlight-grown leaves in comparison with full sunlight- grown leaves (Fig. 2). A similar trend was also noticed for whole chain (H_2O MV) electron transport (Fig. 2).

To locate the possible site of inhibition in the PSII reaction, we followed the DCPIP reduction supported by $MnCl_2$, DPC and NH_2OH, that donate electrons in the PSII reaction (Wydrzynski and Govindjee 1975) in thylakoids of full and 40 % sunlight-grown leaves (Fig. 3). In 40 % sunlight-grown leaves the PSII activity was reduced to about 50 % of that in full sunlight-grown leaves if water or $MnCl_2$ served as elec-

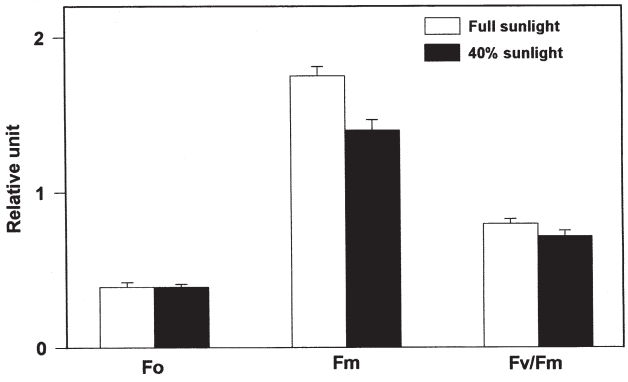

Fig. 1: The relative levels of chlorophyll fluorescence emitted as Fo, Fm and Fv/Fm in the leaves from full and 40 % sunlight-grown leaves. Mean values of five independent experiments, bars denote confidence limits at the 5 % level.

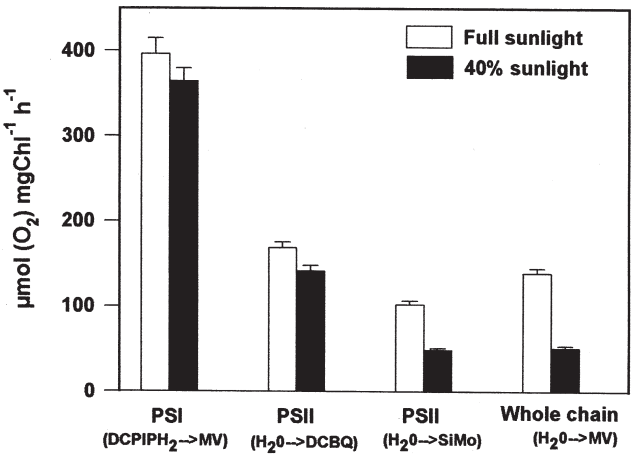

Fig. 2: The rates of whole chain (H_2O MV), PSII (H_2O DCBQ; H_2O SiMo) and PSI ($DCPIPH_2$ MV) electron transport activities in thylakoids isolated from full and 40 % sunlight-grown leaves. Mean values of five independent experiments, bars denote confidence limits at the 5 % level.

Table 1

Leaf pigments, soluble proteins, Rubisco activity, and nitrate reductase activities (in relation to various reference units or with and without KNO_3 fertilization) of leaves collected from full and 40 % sunlight-grown plants. Figures in parentheses are relative reductions or increases with reference to full sunlight-grown leaves. Values are means ± SD (n=5)

Parameters	Full sunlight	40 % sunlight
Chl a [mg.g^{-1} (f.m.)]	1.49 ± 0.06	1.70 ± 0.07 (+14)
Chl b [mg.g^{-1} (f.m.)]	0.53 ± 0.02	0.78 ± 0.03 (+48)
Total Chl [mg.g^{-1} (f.m.)]	2.02 ± 0.10	2.48 ± 0.11 (+23)
Car [mg.g^{-1} (f.m.)]	0.81 ± 0.03	1.05 ± 0.04 (+28)
Chl a/b [mg.g^{-1} (f.m.)]	2.80 ± 0.13	2.20 ± 0.10
Soluble proteins [mg.g^{-1} (f.m.)]	41.20 ± 1.80	30.50 ± 1.50 (-26)
Soluble protein/Chl ratio	20.40 ± 0.90	12.30 ± 0.5
Rubisco [µmol (CO_2) mg^{-1} (protein) h^{-1}]	48.60 ± 2.40	34.00 ± 1.50 (-30)
Nitrate reductase [µmol (NO_2^-) mg^{-1} (f.m.) h^{-1}]	64.22 ± 3.10	29.54 ± 1.30 (-54)
Nitrate reductase [µmol (NO_2^-) mg^{-1} (Chl) h^{-1}]	42.44 ± 2.00	14.85 ± 1.10 (-65)
Nitrate reductase [µmol (NO_2^-) mg^{-1} (f.m.) h^{-1}] -15 mM KNO_3	42.14 ± 1.90	29.00 ± 1.40 (-31)
Nitrate reductase [µmol (NO_2^-) mg^{-1} (f.m.) h^{-1}] +15 mM KNO_3	78.62 ± 3.50	36.24 ± 1.70 (-54)

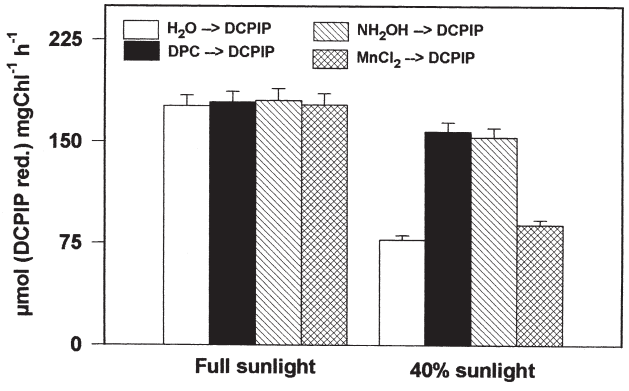

Fig. 3: Effect of various exogenous electron donors on PSII activity (H_2O DCPIP) in thylakoids isolated from full and 40 % sunlight-grown leaves. Mean values of five independent experiments, bars denote confidence limits at the 5 % level.

tron donor. In contrast, a significant restoration of PSII-mediated DCPIP reduction was observed if NH_2OH and DPC were used as electron donors in 40 % sunlight-grown leaves (Fig. 3).

These results agree with measurements obtained by modulated Chl fluorescence with various exogenous electron donors used in thylakoids of full and 40 % sunlight-grown leaves (Tab. 2). The addition of DPC and NH_2OH to thylakoids of 40 % sunlight-grown leaves induced a significant increase of variable fluorescence (Fv). The Fv/Fm ratio also increased from 0.595 to 0.698. In this experiment, the level of Fo was not changed (Tab. 2).

Table 2

Changes in the relative levels of chlorophyll fluorescence emitted as minimal fluorescence (Fo), variable fluorescence (Fv) and the ratio of variable to maximum fluorescence (Fv/Fm) in thylakoids isolated from full sunlight and 40 % sunlight-grown leaves with or without exogenous electron donors. Concentrations of $MnCl_2$, DPC and NH_2OH were 5, 0.5 and 5 mM, respectively. Values are the means ± SD (n=5).

Addition	Fo	Fv	Fv/Fm
Full sunlight			
None	1.9±0.07	5.0±0.19	0.724±0.03
DPC	1.9±0.08	5.4±0.20	0.739±0.04
NH_2OH	1.9±0.07	5.3±0.18	0.736±0.04
$MnCl_2$	1.9±0.06	5.0±0.20	0.724±0.02
40 % sunlight			
None	1.9±0.07	2.8±0.08	0.595±0.02
DPC	1.9±0.05	4.4±0.14	0.698±0.03
NH_2OH	1.9±0.06	4.2±0.10	0.688±0.04
$MnCl_2$	1.9±0.06	3.0±0.06	0.612±0.02

Thylakoid membrane proteins: Since the changes in photosynthetic electron transport activities could be caused primarily by the changes or reorganization of thylakoid components, the polypeptide profiles of full and 40 % sunlight thylakoid membranes were analyzed by SDS-PAGE. A comparison of 40 % sunlight thylakoid membrane polypeptide with those of full sunlight thylakoids indicated a specific loss in the levels of 47, 33, 28-25 and 23 kDa polypeptides (Fig. 4).

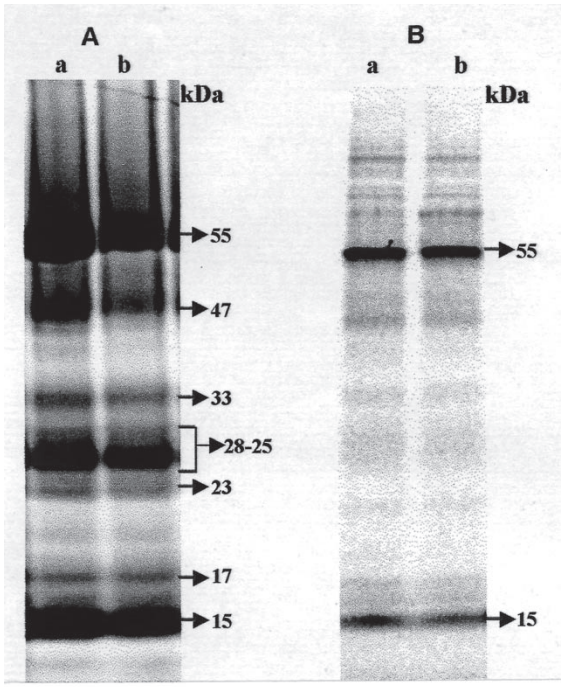

Fig. 4. Coomassie Brilliant-stained polypeptide profiles of thylakoid membranes (**A**) and crude leaf extracts (**B**) isolated from full and 40 % sunlight-grown leaves. Lane a, full sunlight; lane b, 40 % sunlight. Gel lanes were loaded with equal amount of protein (100 µg).

D1 and 33 kDa proteins by immunoblot: The 40 % sunlight induced inhibition of PSII activity in grapevine thylakoids was compared with changes in the relative contents of D1 protein of the PSII reaction center and 33 kDa protein of the water-splitting complex as determined by western blotting followed by quantification by the Bio-Image apparatus (Fig. 5). The relative content of D1 and 33 kDa proteins decreased to 12 and 48 % in 40 % sunlight thylakoids, respectively.

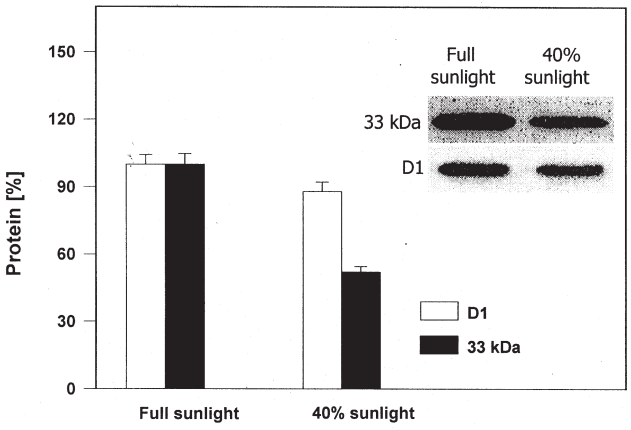

Fig. 5: Degradation of the D1 and 33 kDa proteins in full and 40 % sunlight-grown leaves. Each lane was loaded in equal amounts of Chl (5 µg). Histogram: BioImage densitometrical evaluation. Inset: Western-blot.

Rubisco activity and soluble proteins: When the enzyme activity in crude leaf extracts was expressed on a protein basis, significantly less Rubisco activity was observed in 40 % sunlight-grown

leaves than in full sunlight-grown leaves (Tab. 1). A similar trend was also noticed for soluble proteins (Tab. 1). The contents of 55 (large subunit-LSU) and 15 kDa (small subunit-SSU) polypeptides of Rubisco was marginally reduced in 40 % sunlight-grown leaves (Fig. 4).

Nitrate reductase activity: *In vivo* nitrate reductase activity expressed on a fresh mass basis was decreased by 54 % in 40 % sunlight-grown leaves. When expressed on a Chl unit, the enzyme activities were reduced by 65 % (Tab. 1). The full sunlight-grown leaves incubated with 15 mM KNO_3 tended to have more induced nitrate reductase activity than the 40 % sunlight-grown leaves.

Discussion

Plants acclimated to high irradiance develop alterations at the molecular level when exposed to reduced growth irradiance (Anderson and Andersson 1988). They acclimate by changing their thylakoid membrane composition, the light-harvesting complex, chloroplast ultrastructure and conductances for gas exchange (Boardman 1977, Melis and Harvey 1981, Chow and Hope 1987, Evans 1993, Koesmaryono *et al*. 1998). Higher Chl content and decrease in Chl a/b ratio observed in the 40 % sunlight-grown plants of the present findings agree with those of Björkman *et al*. (1972), Marini and Marini (1983), and Anderson *et al*. (1988). The increase in the Chl content was accompanied by relative increases in the accessory pigment Chl b over that of Chl a as depicted by a decrease of the Chl a/b ratio (Boardman 1977, Lewandowska and Jarvis 1977, Masarovicova and Elias 1981). The Car content showed a similar trend. Total and relative contents of Car remained higher in all leaves under 40 % sunlight compared to full sunlight. Relative increase in the accessory pigments like Chl b and Car are adaptive responses of plants to variable PFD (Anderson *et al*. 1988, Misra 1995). Chl b is a pigment associated with distal antennae of LHCP II, the relative change in this pigment could indicate a change in the distal antenna size (Misra 1995). The increase in the distal antenna size under 40 % sunlight-grown leaves possibly increases the relative radius of solar energy interception in a chloroplast.

Chlorophyll fluorescence induction curves, reflecting photosynthesis and electron transport have characteristic patterns, which undergo changes when the photosynthetic system becomes impaired. They can therefore be used as indicators of damage (Govindjee and Papageorgiou 1971). Full sunlight-grown leaves showed a high PSII activity, measured as the Fv/Fm ratio, while 40 % sunlight-grown leaves showed the lowest Fv/Fm ratio. Fv was reduced markedly in 40 % sunlight-grown leaves while the Fo level was not affected. Reduction of the Fv yield, as shown by many workers, indicates impairment of PSII activity, particularly at the donor site (Klimov *et al*. 1977, Allakhverdiev *et al*. 1987, Setlik *et al*. 1990).

Analysis of various electron transport activities measured by using electron acceptors in thylakoids isolated from full and 40 % sunlight-grown leaves, showed an inhibition of the whole chain of electron transport activity by >60 % in 40 % sunlight-grown leaves; only a marginal effect on PSII-mediated reactions was noticed. Thus 40 % sunlight has an action site(s) in the PSII reaction. Similar large reductions of PSII activity have been reported for low-light-grown plants of *Atriplex* (Boardman *et al*. 1975) and *Picea* (Lewandowska *et al*. 1976). Analysis of electron transport in thylakoids isolated from 40 % sunlight-grown leaves showed that O_2 evolution was significantly inhibited if the electron acceptor used was SiMo, but was not significantly inhibited if the electron acceptor was DCBQ. Since DCBQ is known to accept the electrons directly from Q_A^- (Cao and Govindjee 1990), the rates measured represent the true rate of photochemistry by PSII, not influenced by the PQ pool.

In order to locate the possible site of 40 % sunlight-induced inhibition, we measured PSII-mediated DCPIP reduction in the presence of various artificial exogenous electron donors acting at the oxidizing side of PSII. Among the artificial electron donors tested, DPC and NH_2OH were found to be more effective in restoring the loss of PSII activity in 40 % sunlight-grown leaves. These results were also confirmed by measurement of modulated Chl fluorescence. After addition of DPC and NH_2OH to thylakoids from 40 % sunlight-grown leaves, Fv increased markedly. These results also clearly indicate that 40 % sunlight impaired at the donor side of PSII, perhaps close to the DPC donation side in grapevine leaves.

The most likely explanation for the inactivation of electron transport PSII activity in 40 % sunlight-grown leaves is that the related protein are affected because they are exposed to the thylakoid surface (Seilder 1994). Such reduction of PSII activity in 40 % sunlight-grown leaves was associated with a marked loss of 33 and 23 kDa polypeptides. The extrinsic proteins of 33, 23 and 17 kDa associated with the lumenal surface of the thylakoid membranes are required for optimal functioning of the oxygen evolving machinary (Murata *et al*. 1984, Millner *et al*. 1987, Enami *et al*. 1994). Our results indicate that the significant loss of 33 and 23 kDa polypeptides could be one of the reasons for the significant loss of O_2 evolution capacity in 40 % sunlight-grown leaves.

As shown by the corresponding western blots, a marginal loss of the D1 protein occurs in 40 % sunlight-grown leaves which was accompanied by a significant decrease in the content of 33 kDa protein of the water-splitting system, showing that the whole PSII rapidly degraded under 40 % sunlight condition. A similar phenomenon has already been observed by Schuster *et al*. (1988) under photoinhibitory conditions. These data confirm assumptions that PSII is especially vulnerable to stress conditions (Kyle 1987, Baker 1991).

The 40 % sunlight-grown leaves induced not only losses of extrinsic proteins but there was also a loss of 47 and 28-25 kDa polypeptides in thylakoid membranes which may result from a greater disruption of the PSII complex. This could be a reason for the observed lowering of PSII activity in 40 % sunlight-grown leaves.

Plants grown under 40 % sunlight condition have relatively low levels of soluble proteins and soluble protein/chlorophyll ratios. This concurs with similar reports (Bordman 1977, Bjorkman 1981, Givnish 1988, Burkey *et al*. 1997). The low level of soluble proteins in 40 % sunlight-grown leaves might be due to a decrease of Rubisco synthe-

sis, the major soluble protein in leaves. A marked loss of Rubisco activity was observed in 40 % sunlight-grown leaves. This is mainly due to an inhibition of protein synthesis in grapevine leaves. It is also suggested that the carboxylating enzymes are not fully activated at low light intensity and that the degree of activation will regulate the flux of carbon through the photosynthetic pathway (USUDA *et al.* 1985). This is supported by SDS-PAGE analysis of crude leaf extracts of Rubisco proteins showing a marginal loss of 55 (large subunit-LSU) and 15 kDa (small subunit-SSU) polypeptide in 40 % sunlight-grown leaves. This marginal loss of 55 kDa and 15 kDa polypeptides is one of the reasons for the loss of Rubisco activity in 40 % sunlight-grown leaves.

Conclusion

To the best of our knowledge, for the first time the present results demonstrate that field-grown grapevine plants grown under full and 40 % sunlight have different leaf pigment composition, ribulose-1,5-bisphosphate carboxylase, soluble proteins, nitrate reductase and photosynthetic activities of the thylakoids. In addition, 40 % sunlight impaired on the donor side of PSII in grapevine leaves. This is due to: 1) a marked inhibition of PSII activity if SiMo was used as electron acceptor, 2) an artificial exogenous donors DPC and NH_2OH significantly restored the loss of PSII activity, 3) the level of Fo did not changed, 4) the content of 33 and 23 kDa polypeptides are markedly reduced, and 5) the level of 33 kDa protein was significantly reduced in 40 % sunlight-grown leaves.

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References

ALLAKHVERDIEV, S. I.; SETLIKOVA, E.; KLIMOV, V. V.; SETLIK, I.; 1987: In phototoinhibited photosystem II particles pheophytin in photoreduction remains unimpaired. FEBS Lett. **226**, 186-190.

ANDERSON, J. M.; 1986: Photoregulation of the composition, function and structure of thylakoid membranes. Ann. Rev. Plant Physiol. **37**, 93-136.

ANDERSON, J. M.; ANDERSSON, B.; 1988: The dynamic photosynthetic membrane and regulation of solar energy conversion. Trends Biochem. Sci. **13**, 351-355.

ANDERSON, J. M.; CHOW, W. S.; GOODCHILD, D. J.; 1988: Thylakoid membranes organization in sun/shade acclimation. Aust. J. Plant. Physiol. **15**, 11-26.

BAKER, N. R.; 1991: A possible role for photosystem II in environmental perturbations of photosynthesis. Physiol. Plant **81**, 563-570.

BERTHHOLD, D. A.; BABCOCK, G. T.; YOCUM, C. A.; 1981: Highly resolved O_2 evolving photosystem II preparation from spinach thylakoid membranes. FEBS Lett. **134**, 231-234.

BJÖRKMAN, O.; 1981: Responses to different quantum flux densities. In: A. PIRSON, M. H. ZIMMERMANN (Eds): Encyclopedia of Plant Physiology, 57-107. Springer-Verlag, Berlin.

BJÖRKMAN, O.; LUDLOW, M.; MORROW, P.; 1972: Photosynthetic performance of two rain forest species in their native habitat and analysis of their gas exchange. Carnegie Inst. Washington Yearb. **77**, 94-102.

BJÖRKMAN, O.; TROUGHTON, J.; NOBS, M. A.; 1973: Photosynthesis in relation to leaf structure. Brook Haven Symp. Biol. **25**, 206-226.

BOARDMAN, N. K.; 1977: Comparing photosynthesis of sun and shade plants. Ann. Rev. Plant Physiol. **28**, 355-377.

BOARDMAN, N. K.; BJORKMAN, O.; ANDERSON, J. M.; GOODCHILD, D. J.; GRIMME, L. H.; THORNE, S. W.; 1975: Photosynthetic adaptation of higher plants to light intensity: Relationship between chloroplast structure, composition of the photosystems and photosynthetic rates. In: M. AVRON (Ed.): Third. Int. Congr. Photosynthesis, 1809-1827. Elsevier, Amsterdam.

BRADFORD, M. M.; 1976: A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**, 248-254.

BURKEY, K. O.; WILSON, R. F.; WELLS, R.; 1997: Effects of canopy on the lipid composition of soybean leaves. Physiol. Plant. **101**, 591-598.

CAO, J.; GOVINDJEE; 1990: Chlorophyll transient as an indicator of active and inactive photosystem II in thylakoid membranes. Biochim. Biophys. Acta **1015**, 180-188.

CHARLES-EDWARDS, D. A.; LUDWIG, L. J.; 1975: The basis of expression of leaf photosynthesis activities. In: R. MARCELLE, (Ed.): Environmental and Biological Control of Photosynthesis, 36-51. Martinus Nijhoff/W. Junk Publ., Hague.

CHOW, W. S.; HOPE, A. B.; 1987: The stoichiometries of supramolecular complexes in thylakoid membranes from spinach chloroplasts. Aust. J. Plant. Physiol. **14**, 21-28.

CLARKSON, D. T.; 1986: Regulation of absorption and release of nitrate by plant cells. A review of current ideas and methodology. In: H. LAMBERS, J. J. NEETESON, I. STULEN (Eds.): Fundamental, Ecological and Agricultural Aspects of Nitrogen Metabolism in Higher Plants, 3-27. Martinus Nijihoff Publ., Dordrecht.

ENAMI, I.; KITAMURA, M.; TOMO, T.; ISOKAWA, Y.; OHTA, H.; KATOH, S.; 1994: Is the primary cause of thermal inactivation of O_2 evolution in spinach PSII membranes release of the extrinsic 33 kDa protein or of Mn? Biochim. Biophys. Acta **1186,** 52-58.

EVANS, J. R.; 1988: Acclimation by thylakoid membranes to growth irradiance and the partitioning of nitrogen between soluble and thylakoid proteins. Aust. J. Plant Physiol. **15**, 93-106.

EVANS, J. R.; 1993: Photosynthetic acclimation and nitrogen partitioning within a lucerne canopy. I. Canopy characteristics. Aust. J. Plant. Physiol. **20**, 55-67.

GIVNISH, T. J.; 1988: Adaptation of sun and shade: A whole plant perspective. In: J. R. EVANS, VON CAEMMER, W. E. ADAMS (Eds.): Ecology of Photosynthesis in Sun and Shade, 63-92. CSIRO, Melbourne.

GOVINDJEE; PAPAGEORGIOU, G.; 1971: Chlorophyll fluorescence and photosynthesis: Fluorescence transients. In: A. C. GIESE (Ed.): Phytophysiology, 1-46. Academic Press, New York.

HUNTER, J. J,; VISSER, J. H.; 1988: The effect of partial defoliation, leaf position and developmental stage of the vine on leaf chlorophyll concentration in relation to the photosynthetic activity of *Vitis vinifera* L. cv. Cabernet Sauvignon. S. Afr. J. Enol. Vitic. **9**, 9-15.

JAWORSKI, E. G.; 1971: Nitrate reductase assay in intact plant tissues. Biochem. Biophys. Res. Commun. **43**, 1274-1279.

KLIMOV, V. V.; KLEVANIK, A. V.; SHURAVLOV, V. A.; KRASNOVSKY, A. A.; 1980: Reduction of pheophytin in the primary light reaction of photosystem II. FEBS Lett. **82**, 183-186.

KOESMARYONO. Y.; SUGIMOTO, K.; ITO, D.; HASEBA, T.; SATO, T.; 1998: Photosynthetic and transpiration rates of soybean as affected by different irradiances during growth. Photosynthetica **35**, 573-578.

KRAUSE, G. H.; WEIS, E.; 1991: Chlorophyll fluorescence and photosynthesis: The basics. Ann. Rev. Plant Physiol. Plant. Mol. Biol. **42**, 313-349.

KYLE, D. J.; 1987: The biochemical basis for photoinhibition of photosystem II. In: D. J. KYLE, C. B. OSMOND, C. J. ARNTZEN (Eds.): Topics of Photosynthesis, 197-226. Elsevier, Amsterdam.

LAEMMLI, U. K.; 1970: Clevage of structural proteins during the assembly of the head bacteriophage T_4. Nature **227**, 680-685.

LEWANDOWSKA, M.; HART, J. W.; JARVIS, P. G.; 1976: Photosynthetic elec-

tron transport in plants of Sitka spruce subjected to different light environments during growth. Physiol. Plant. **37**, 269-274.

Lewandowska, M.; Jarvis, P. G.; 1977: Changes in chlorophyll and carotenoid content, specific leaf area and dry weight fraction in Sitka spruce in response to shading and season. New Phytol. **79**, 247-256.

Lichtenthaler, H. K. 1987. Chlorophylls and carotenoids, the pigments of photosynthetic theromembranes. Meth. Enzymol. **148**, 350-382.

Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J.; 1951: Protein measurement with Folin phenol reagent. J. Biol. Chem. **193**, 265-275.

Marini, R. P.; Marini, M. C.; 1983: Seasonal changes in specific leaf weight, net photosynthesis and chlorophyll content of peach leaves as affected by light penetration and canopy position. J. Am. Soc. Hortic. Sci. **108**, 609-613.

Masarovicova, E.; Elias, P.; 1981: Chlorophyll content in leaves in an oak-hornbeam forest. 2. Shrub species. Photosynthetica **15**, 16-20.

McClenden, J. H.; McMillen, G. G.; 1982: The control of leaf morphology and the tolerance to shade by woody plants. Bot. Gaz. **143**, 79-83.

Melis, A.; Harvey, G. W.; 1981: Regulation of photosystem stoichiometry clorophyll a and chlorophyll b content and relation to chloroplast ultrastructure. Biochim. Biophys. Acta **637**, 138-145.

Millner, P. A.; Gogel, G.; Barbar, J.; 1987: Investigation of the spatial relationship between photosystem II polypeptides by reversible crosslinking and diagonal electrophoresis, Photosynth. Res. **13**, 185- 198.

Misra, M.; 1995: Growth, photosynthetic pigment content and oil yield of Pogostemon cablin grown under sun and shade conditions. Biol. Plant. **37**, 319-323.

Murata, N.; Miyao, M.; Omata, T.; Matsunami, H.; Kuwabara, T.; 1984: Stoichiometry of components in the photosynthetic O_2 evolution system of photosystem II particles prepared with Triton X-100 from spinach chloroplasts. Biochim. Biophys. Acta **765,** 363-369.

Nedunchezhian, N.; Kulandaivelu, G.; 1991: Effect of enhanced radiation on ribulose-1,5-bisphosphate carboxylase in leaves of *Vigna sinensis* L. Photosynthetica **25**, 231-435.

Nedunchezhian, N.; Morales, F.; Abadia, A.; Abadia, J.; 1997: Decline in photosynthetic electron transport activity and changes in thylakoid protein pattern in field grown iron deficient Peach (*Prunus persica* L.). Plant Sci. **129**, 29-38.

Schuster, G.; Timberg, R.; Ohad, I.; 1988: Turnover of thylakoid photosystem II proteins during photoinhibition the ninth of *Chlamydomonas reinhardtii*, Eur. J. Biochem. **177**, 403-410.

Seidler, A.; 1994: Expression of the 23 kDa protein from the oxygen-evolving complex of higher plants in *Escherichia coli.* Biochim. Biophys. Acta **1187,** 73-79.

Senger, H.; Bauer, B.; 1987: The influence of light quality on adaptation and function of the photosynthetic apparatus. Photochem. Photobiol. **45**, 939-946.

Setlik, I.; Allakhverdiev, S. I.; Nedbal, L.; Setlikova, E.; Klimov, V. V.; 1990: Three types of photosystem II photoinactivation. Photosynth. Res. **23**, 39-48.

Wydrzynski, T.; Govindjee; 1975: A new site of bicarbonate effect in photosystem II of photosynthesis: Evidence from chlorophyll fluorescence transients in spinach chloroplasts. Biochim. Biophys. Acta **387**, 403-408.

Usuda, H.; Ku, S. B. M.; Edwards, G. E.; 1985: Influence of light intensity during growth on photosynthesis and activity of several key photosynthetic enzymes in a C_4 plant (*Zea mays*). Physiol. Plant. **63**, 65-67.

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