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

M. BERTAMINI and N. NEDUNCHEZHIAN

Istituto Agrario di San Michele all’ Adige, 38010 San Michele all’ Adige (TN), Italia

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 NH₄OH, significantly restored the loss of PSI activity in 40 % sunlight-grown leaves. The same results were obtained when Fv/Fm was evaluated by chlorophyll fluorescence measurements. The marked loss of PSI 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.

Key words: chlorophyll fluorescence, electron transport, donor side, nitrate reductase, photosystem.

Abbreviations: 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; Rubisc = ribulose-1,5-bisphosphate carboxylase, SDS-PAGE = sodium dodecyl sulfate-polyacrylamide gel electrophoresis, SiMo = siliconomolybdate.

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 (BJORKMAN et al. 1973, MCCLENDEN and McMILLENN 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 Q₉ 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 VISER 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 VISER (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

Plant growth: 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
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 N₂ 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 measured at 20 kHz with a 1 s 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 MgCl₂, 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 Bertoldi et al (1981). Whole chain electron transport (H₂O – MV) and partial reactions of photosynthetic electron transport mediated by PSII (H₂O – DCBQ; H₂O – SiMo) and PSI (DCPPIP₇ – MV) were measured as described by Nedunchezian 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 MgCl₂, 5 mM NH₄Cl and 100 mM sucrose supplemented with 500 µM DCBQ and 200 µM SiMo₆.

**DCPIP photoreduction measurements:** DCPIP reduction was determined as the decrease in absorbance at 590 nm using a Hitachi S57 spectrophotometer. The reaction mixture (3 ml) contained 20 mM Tris-HCl, pH 7.5, 5 mM MgCl₂, 10 mM NaCl, 100 mM sucrose, 100 mM DCPIP and thylakoids equivalent to 20 µg of Chl. Where mentioned, the concentrations of MnCl₂, DPC and NH₄OH 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 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 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 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 MgCl₂, 1 mM EDTA, 10 mM 2-mercaptoproethanol, 10 mM PMSF in the presence of liquid nitrogen. Homogenates were filtered 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 MgCl₂, 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 Nedunchezian and Kulandaiavelu (1991).

**Nitrate reductase activity:** Leaves (100 mg) were suspended in a glass vial containing 5 ml of the assay medium consisting of 100 mM KH₂PO₄-KOH, pH 7.0, 100 mM KNO₃, 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 NO₂⁻ 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 WIEs 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 DCPIP, 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 H2O DCBQ and H2O 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 (H2O MV) electron transport (Fig. 2).

To locate the possible site of inhibition in the PSII reaction, we followed the DCPIP reduction supported by MnCl2, DPC and NH2OH, 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 MnCl2 served as elec-

Table 1
Leaf pigments, soluble proteins, Rubisco activity, and nitrate reductase activities (in relation to various reference units or with and without KNO3 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)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Full sunlight</th>
<th>40% sunlight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chl a [mg·g⁻¹(f.m.)]</td>
<td>1.49±0.06</td>
<td>1.70±0.07 (+14)</td>
</tr>
<tr>
<td>Chl b [mg·g⁻¹(f.m.)]</td>
<td>0.53±0.02</td>
<td>0.78±0.03 (+48)</td>
</tr>
<tr>
<td>Total Chl [mg·g⁻¹(f.m.)]</td>
<td>2.02±0.10</td>
<td>2.48±0.11 (+23)</td>
</tr>
<tr>
<td>Car [mg·g⁻¹(f.m.)]</td>
<td>0.81±0.03</td>
<td>1.05±0.04 (+28)</td>
</tr>
<tr>
<td>Chl a/b [mg·g⁻¹(f.m.)]</td>
<td>2.80±0.13</td>
<td>2.20±0.10</td>
</tr>
<tr>
<td>Soluble proteins [mg·g⁻¹(f.m.)]</td>
<td>41.20±1.80</td>
<td>30.50±1.50 (-26)</td>
</tr>
<tr>
<td>Soluble protein/Chl ratio</td>
<td>20.40±0.90</td>
<td>12.30±0.5</td>
</tr>
<tr>
<td>Rubisco [µmol (CO₂) mg⁻¹(protein) h⁻¹]</td>
<td>48.60±2.40</td>
<td>34.00±1.50 (-30)</td>
</tr>
<tr>
<td>Nitrate reductase [µmol (NO₃⁻) mg⁻¹(f.m.) h⁻¹]</td>
<td>64.22±3.10</td>
<td>29.54±1.30 (-54)</td>
</tr>
<tr>
<td>Nitrate reductase [µmol (NO₃⁻) mg⁻¹(Chl) h⁻¹]</td>
<td>42.44±2.00</td>
<td>14.85±1.10 (-65)</td>
</tr>
<tr>
<td>Nitrate reductase [µmol (NO₃⁻) mg⁻¹(f.m.) h⁻¹] with 15 mM KNO₃</td>
<td>42.14±1.90</td>
<td>29.00±1.40 (-31)</td>
</tr>
<tr>
<td>Nitrate reductase [µmol (NO₃⁻) mg⁻¹(f.m.) h⁻¹] with 15 mM KNO₃</td>
<td>78.62±3.50</td>
<td>36.24±1.70 (-54)</td>
</tr>
</tbody>
</table>
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.

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 NH₂OH 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>
<thead>
<tr>
<th>Addition</th>
<th>Fo ± SD</th>
<th>Fᵥ ± SD</th>
<th>Fᵥ/Fm ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full sunlight</td>
<td>None</td>
<td>1.9 ± 0.07</td>
<td>5.0 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>DPC</td>
<td>1.9 ± 0.08</td>
<td>5.4 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>NH₂OH</td>
<td>1.9 ± 0.07</td>
<td>5.3 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>MnCl₂</td>
<td>1.9 ± 0.06</td>
<td>5.0 ± 0.20</td>
</tr>
<tr>
<td>40% sunlight</td>
<td>None</td>
<td>1.9 ± 0.07</td>
<td>2.8 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>DPC</td>
<td>1.9 ± 0.05</td>
<td>4.4 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>NH₂OH</td>
<td>1.9 ± 0.06</td>
<td>4.2 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>MnCl₂</td>
<td>1.9 ± 0.06</td>
<td>3.0 ± 0.06</td>
</tr>
</tbody>
</table>

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).

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. 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.

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. Mean values of five independent experiments, bars denote confidence limits at the 5% level.
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 KNO₃ 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, Koemaryono 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 Bjorkman 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 F0 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 PSI-mediated reactions was noticed. Thus 40 % sunlight has an action site(s) in the PSII reaction. Similar large reductions of PSI 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 O₂ 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 O₂ (Cao andGovindjee 1990), the rates measured represent the true rate of photochemistry by PSI, 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 NH₂OH 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 NH₂OH 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 luminal surface of the thylakoid membranes are required for optimal functioning of the oxygen evolving machinery (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 O₂ 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 PSI 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 synthet-
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 NH₂OH significantly restored the loss of PSII activity, 3) the level of Fo did not change, 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.

Acknowledgements

This work was in part supported by a grant of the Provincia Autonoma of Trento and National Council of Research (CNR): project "Analisi e Ricerche per il sistema Agri-Industriale" sub-project "Prometavit".

References


KLIMOV, V. V.; KLEVANIK, A. V.; SHAYRALEV, V. V.; KLEASNOVSKY, A. A.; 1980: Reduction of phophoeyn in the primary light reaction of photosystem II. FEBS Lett. 82, 183-186.


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.


Received July 17, 2002