BIOLOGIA PLANTARUM 48 (1): 149-152, 2004

BRIEF COMMUNICATION

Photosynthetic responses for *Vitis vinifera* plants grown at different photon flux densities under field conditions

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Abstract

In grapevine (*Vitis vinifera* L.) leaf chlorophyll (Chl) *a* and Chl *b* and carotenoid contents were higher in plants grown at low photon flux densities (PFD) than in those grown at medium and high PFD. The highest Chl *a* variable to maximum fluorescence ratio F_v/F_m was observed in plants grown at medium PFD while the minimum fluorescence F_0 was highest in those at high PFD. In isolated thylakoids, both high and low PFD caused marked inhibition of whole chain and photosystem 2 (PS2) activities. The artificial exogenous electron donor diphenyl carbazide significantly restored the loss of PS2 activity in low PFD leaves.

Additional key words: carotenoids, chlorophyll, donor side, electron transport, grapevine, photosystems 1 and 2.

Acclimation of the photosynthesis to photon flux density (PFD) has been of long-standing interest. The differences in ultrastructure as well as biochemical and physiological properties between leaves of plants grown in full sun and of those grown in deep shade have been well documented (*e.g.,* Lichtenthaler *et al*. 1984, Anderson *et al*. 1988, Evans *et al*. 1993, Yin and Johnson 2000). Variation in radiation quality and duration or irradiance cause significant change in pattern of leaf growth and senescence. Plants grown at high irradiance have small leaf size and dry mass, and low water content, but high chlorophyll (Chl) *a/b* ratio and sun type chloroplasts (Meier and Lichtenthaler 1981, Lichtenthaler *et al*. 1984).

Grapevine is normally planted at densities that result in canopy closure. During the development of a closed grapevine canopy, many leaves expand under full sun, but later function in extreme shade following the development of leaves at higher nodes. The extreme shade conditions can induce rapid senescence of lower canopy leaves several weeks in advance of senescence of the whole plant (Secor *et al*. 1984, Wells 1991). The second type of response is acclimation of photosynthesis in shaded leaves that remain on the plant until senescence. The grapevine canopy consists of leaves of different ages, which are subjected to variable irradiances during the entire growth season (Hunter and Visser 1988). According to Boardman (1977) a leaf photosynthetic productivity is primarily governed by its position in the plant canopy. In the present paper, we report the effect of photon flux densities (PFD) on the changes in the leaf pigments and photosynthetic activities in field grown grapevine leaves.

Leaves were collected from a selected 10-years-old grapevine (*Vitis vinifera* L.) grown under field on training system with upright growing shoots (Cordon Royet) condition in the Istituto Agrario di San Michele all' Adige, Italy. In order to simplify the experimental procedure, we classified the leaves into three groups according to the mean irradiation they received on the leaf surface. This was 200, 850, and 1 750 μmol m^{-2} s^{-1} for low, moderate (control) and high PFD, respectively. The maximum temperature was 32 - 33 °C during the

Received 11 September 2003, *accepted* 7 January 2004.

Abbreviations: Car - carotenoids; Chl - chlorophyll; DCBQ - 2,6-dichloro-*p*-benzoquinone; DCPIP - 2,6-dichlorophenol indophenol; DPC - diphenyl carbazide; E - transpiration rate; F_0 - minimum fluorescence; F_v - variable fluorescence; g_s - stomatal conductance; MV - methyl viologen; PFD - photon flux density; P_N - leaf net photosynthetic rate; PS - photosystem; SiMo - silicomolybdate.

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

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experimental period. Irradiance and temperature were measured as in Iacono *et al*. (1994). The amounts of chlorophyll (Chl) and carotenoids (Car) were determined spectrophotometrically by the method of Lichtenthaler (1987). Measurements of leaf net photosynthetic rate (P_N), stomatal conductance (g_s) and transpiration rate (E) of leaves were taken at 11:00 with a portable photosynthesis system *(LI-6200, LI-COR*, Lincoln, USA).

Modulated Chl fluorescence in leaves was measured on leaf discs using a *PAM 2000* fluorometer (*H. Walz*, Effeltrich, FRG). F_0 was measured by switching on the modulated irradiation of 0.6 kHz; PFD 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".

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 photosystem (PS) 2 (H_2O → DCBQ; H_2O → SiMo) and PS1 ($DCPIPH_2$ → MV) were measured as described by Nedunchezhian *et al*. (1997). Thylakoids were suspended at 10 μg (Chl) cm^{-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 500 μM DCBQ and 200 μM SiMo. The rate of DCPIP photoreduction was determined by following the decrease in absorbance at 590 nm using a *Hitachi 557* (Tokyo, Japan) spectrophotometer. As a donor for PS2, 1 mM DPC was added.

The primary objective was to study the effect of PFD on leaf pigments and photosynthesis of field grown grapevine (*Vitis vinifera* L.) leaves. The contents of Chl and Car were significantly decreased in high PFD leaves (Table 1). The reduction in Chl contents was largely exhibited through the decay of Chl *a* and Chl *b* and high PFD probably enhanced the chlorophyllase activity in grapevine leaves. An increase of Car/Chl ratio and decrease of Chl *a/b* ratio was also noticed in high PFD leaves (Table 1). This was due to the relatively faster decrease of Chl than Car. In contrast, Chl and Car contents were increased under low PFD (Table 1). Increase in Chl content was reported in other plants adapted to low PFD (Anderson *et al.* 1988). This increase was accompanied with relative increase in content of the accessory pigment Chl *b* over that of Chl *a* depicted by a decrease in Chl *a/b* ratio (Table 1).

The low and high PFD grown leaves had significantly lower P_N than the moderate PFD grown leaves (Table 1). As much as 42 and 76 % was P_N decrease in low and high PFD leaves, respectively. Decreased P_N was closely related to g_s which was also decreased by 10 and 32 %, respectively, in low and high PFD grown leaves. Similar trend was also observed for *E* in low and high PFD leaves (Table 1).

To obtain information on PS2 activity, F_v/F_m, which reflects the quantum yield of PS2 photochemistry (Krause and Weis 1991), was determined. F_v/F_m was significantly decreased in low and high PFD leaves (Table 1). The effect of low PFD was prominent on the variable fluorescence (F_v) without increase of F_0 (Table 1).

The whole chain electron transport was inhibited by

Table 1. Changes in contents of leaf pigments, gas exchange rate, chlorophyll fluorescence, and electron transport activities in leaves of plants grown at low, medium and high PFD. Means ± SE of 5 replicates of each experiment.

Parameters		Low PFD	Medium PFD	High PFD
Pigments	Chl *a*	1.38 ± 0.06	1.21 ± 0.07	0.78 ± 0.03
[g kg^{-1}(f.m.)]	Chl *b*	0.58 ± 0.02	0.47 ± 0.02	0.26 ± 0.01
	Chl *a+b*	1.96 ± 0.96	1.68 ± 0.08	1.04 ± 0.05
	Car	0.81 ± 0.04	0.79 ± 0.03	0.71 ± 0.03
	Chl *a/b*	2.39	2.60	3.03
	Car/Chl	0.41	0.47	0.68
Gas exchange rate	P_N	5.78 ± 0.26	10.05 ± 0.50	2.44 ± 0.11
[mmol m^{-2} s^{-1}]	g_s	55.44 ± 2.60	61.61 ± 3.10	41.58 ± 1.90
	E	2.64 ± 0.13	3.05 ± 0.10	2.13 ± 0.10
Chl fluorescence	F_0	0.38 ± 0.01	0.38 ± 0.01	0.40 ± 0.02
	F_v	0.81 ± 0.04	1.58 ± 0.07	1.00 ± 0.05
	F_v/F_m	0.68 ± 0.03	0.81 ± 0.04	0.71 ± 0.04
Electron transport	H_2O → MV	81.00 ± 4.00	142.20 ± 7.10	92.40 ± 4.40
[mmol(O_2) kg^{-1}(Chl) s^{-1}]	H_2O → DCBQ	150.20 ± 7.40	168.80 ± 8.40	114.50 ± 5.40
	H_2O → SiMo	63.80 ± 3.10	106.40 ± 5.10	101.10 ± 4.90
	H_2O → DCPIP	90.60 ± 4.60	156.30 ± 7.80	103.20 ± 5.10
	DPC → DCPIP	140.40 ± 7.00	158.50 ± 7.20	109.40 ± 4.90
	$DCPIPH_2$ → MV	240.90 ± 12.0	261.90 ± 12.7	251.40 ± 12.5

43 and 35 % in low and high PFD leaves, respectively (Table 1). However, the PS1 activity was much less diminished. Low and high PFD leaves did not produce any significant change in the rate of PS1 activity. Similar large reduction in PS2 activity was reported in low PFD grown plants of *Atriplex* (Boardman *et al*. 1975) and *Picea* (Lewandowska *et al*. 1976). The analysis of electron transport activities in thylakoids isolated from high PFD leaves showed that the O_2 evolution was inhibited markedly when the electron acceptor used was DCBQ, but not SiMo (Table 1), mainly due to high PFD induced changes on the reducing side of PS2 due to photoinhibition. This may be supported by an increase in F_0 shown by Asada *et al*. (1992) and Endo *et al*. (1998). In contrast, in thylakoids isolated from low PFD leaves, the rate of PS2 activity was lower with SiMo than with DCBQ. This shows that the donor side was more impaired than the acceptor side of PS2. Also F_v was reduced markedly without increase in F_0. This is characteristic for inhibition of donor side of PS2 (Allakhverdiev *et al*. 1987, Šetlík *et al*. 1990). A relationship between F_v/F_m and PS2 electron transport activity in thylakoids isolated from photoinhibited leaves was also shown by Somersalo and Krause (1990) and Schnettger *et al*. (1994).

DPC as artificial electron donor for PS2 markedly restored the loss of PS2 activity in low PFD leaves (Table 1). This is due to water-oxidizing system being sensitive to low PFD in grapevine leaves. In contrast, using DPC in high PFD leaves did not restore the loss of PS2 activity. Hence high PFD induced changes only on the acceptor side of PS2 in grapevine leaves (Asada *et al*. 1992, Hong and Xu 1999). Similar observations were found for field grown *Schefflera arboricola* leaves adapted to different radiation environments (Schiefthaler *et al*. 1999).

Our results suggest that low PFD induced changes on the donor side of PS2 and induced senescence or ageing in grapevine leaves. This was probably due to: *a*) more marked loss of PS2 activity by using electron acceptor SiMo than DCBQ, *b*) F_v was reduced markedly without increase in F_0, and *c*) markedly restored loss of PS2 activity by using the electron donor DPC. In contrast, high PFD induced changes on the acceptor side of PS2 due to photoinhibition suggested by *a*) significant loss of PS2 activity by using electron acceptor DCBQ instead of SiMo, *b*) significant increase of F_0, and *c*) DPC did not restore the loss of PS2 activity. Also grapevine plants grown under different irradiances have different leaf pigment contents.

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