RESEARCH ARTICLE

Photosynthesis and cell respiration modulated by water deficit in grapevine (*Vitis vinifera* L.) cv. Cabernet Sauvignon

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ABSTRACT

Winegrape productivity and quality has been related to the regulated deficit irrigation, with important implications for the plant bioenergetics. When water deficit is imposed to grapevine plants, alterations in overall photosynthesis and cell respiration are observed. The aim of this study was to assess the modulations promoted by water stress on photosynthesis and respiration in leaves of the cv. Cabernet Sauvignon (cv. CS) for better understanding the physiological responses related to its drought tolerance and quality improvement under water deficit. For this purpose, measurements of photosynthetic efficiency, leaf water potential, gas exchange and O_2 consumption were carried out. Leaf water potential, photosynthesis, stomatal conductance, transpiration and internal carbon concentration were significantly reduced upon stress, suggesting that plants of cv. CS present higher water use efficiency (A_N/E) and lower carboxylative capacity (A_N/Ci) under this condition. On the other hand, cell respiration increased more than 70 % as estimated by the increase of O_2 consumption measured 12 days after suspension of irrigation. Most of this effect was related to a four-fold increase of the mitochondrial alternative oxidase (AOX) activity. These data indicate a key role for the AOX pathway in the physiological responses of grapevines to water deficit, and it implies that analyses of the AOX activation patterns should be useful for programs aiming to improve the consistency of fruit production and quality of winegrape cultivars by regulated deficit irrigation.

Keywords: *Vitis vinifera*, drought tolerance, irrigation management, respiratory chain regulation, plant bioenergetics.

MODULAÇÕES NA FOTOSSÍNTESE E RESPIRAÇÃO CELULAR RELACIONADAS À DEFICIÊNCIA HÍDRICA EM VIDEIRA (*VITIS VINIFERA* L.) CV. CARBERNET SAUVIGNON

Resumo – A qualidade e produtivade das uvas viníferas têm sido relacionadas a privação regulada de irrigação, o que sugere implicações importantes para a bioenergética da planta. Quando submetidas ao déficit hídrico as videiras apresentam alterações globais na fotossíntese e na respiração. O objetivo deste trabalho foi avaliar as modulações promovidas pelo estresse hídrico na fotossíntese

e respiração em folhas da cv. Cabernet Sauvignon (cv. CS) visando o melhor entendimento das respostas fisiológicas relacionadas com sua tolerância à seca e melhoria da qualidade sob estresse hídrico. Para tanto, mensurou-se a eficiência fotossintética, o potencial hídrico foliar, as trocas gasosas e o consumo de oxigênio. O potencial hídrico foliar, fotossíntese, condutância estomática, transpiração e concentração interna de carbono foram significativamente reduzidas com o estresse, sugerindo que as plantas da cv. CS apresentam alta eficiência no uso da água (A_N/E) e baixa eficiência carboxilativa (A_N/Ci) sob essa condição. Por outro lado, a respiração celular estimada pelo consumo de O_2 aumentou mais que 70 % aos doze dias após a suspensão da irrigação, sendo a maior parte desse efeito relacionada a um aumento de quatro vezes na atividade da oxidase alternativa mitocondrial (AOX). Estes dados indicam um papel chave para a AOX nas respostas fisiológicas das videiras ao estresse hídrico, e implica que análises dos padrões de ativação da AOX podem ser úteis para programas que objetivam melhorar a consistência da produção e qualidade dos frutos pela suspensão controlada da irrigação em uvas viniferas.

Palavras-chaves: *Vitis vinifera*, tolerância à seca, manejo irrigação, regulação cadeia respiratória, bioenergética vegetal.

INTRODUCTION

Water scarcity is considered as the main environmental factor limiting plant growth and yield worldwide (Boyer, 1982; Chaves et al., 2003). Drought has adverse effects on plant growth, affecting mainly leaf and root growth, stomatal conductance, photosynthetic rate and biomass gain (Blum, 1998). Changes in plant growth elicited by low water availability have also been related to modulations of the plant cell carbon metabolism, which are dependent on the balance between photosynthesis and respiration. Although photosynthesis may decrease up to 100 % becoming completely impaired under severe drought, respiration rate may either increase (Bartoli et al., 2005; Shugaeva et al., 2007) or decrease (Huang and Fu, 2000; Galmes et al., 2007). Respiration is an essential metabolic process that generates not only ATP but several other metabolites that are used in many synthetic processes essential for growth and maintenance of the cell homeostasis, including under stress conditions (MacCabe et al., 2000; Bartoli et al., 2000).

A special feature of plant cell respiration is the presence of an alternative pathway which drains electrons from the ubiquinone pool without involvement of the cytochrome oxidase (Brownleader et al., 1997). The mitochondrial alternative oxidase (AOX) apparently reduces molecular oxygen to water in a single four-electron transfer step (Day et al., 1991; Moore and Siedow, 1991). This alternative pathway is nonphosphorylating, resistant to cyanide and antimicine and inhibited by salicilhydroxamic acid (SHAM) and n-propyl gallate (Schonbaum et al., 1971; Siedow and Grivin, 1980). It has been the focus of many studies in plant respiratory metabolism under several environmental stresses, mainly because an increased AOX capacity might contribute to controlling the formation of reactive oxygen species (ROS) (Wagner, 1995; Popov et al., 1997; Maxwell et al., 1999; Umbach et al., 2005). At least part of the drought effects on plant physiology is related to ROS formation, such as superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxil radicals ($^{\cdot}OH$) and singlet oxygen ($^1O_2$) (Li and Staden, 1998). These ROS may initiate destructive oxidative process such as lipid peroxidation, chlorophyll bleaching, protein oxidation, and damage to nucleic acids (Scandalios, 1993).

Water stress invariably decreases the photosynthetic rate and the intensity of this effect influences the capacity of different species to cope with the drought, which also depends on the duration of stress and plant genetic background (Kaiser, 1987; Chaves, 1991, Chaves et al., 2002). Generally, when respiration rate decreases upon drought conditions the photosynthesis and growth requirements are further affected. Nevertheless, this behavior seems to be somewhat species dependent, and respiratory rates can also increase, particularly under severe drought (Gashghaie et al., 2001; Flexas et al., 2005).

Despite of the evidence showing that *V. vinifera* is not only a drought tolerant species, but also its productivity and quality could be improved under regulated water deficit, only few studies have examined the effects of water stress on grapevines respiration. This issue was investigated in the present work in order to shed light on the bioenergetics and gas exchange modulations in *Vitis vinifera* L. cv. Cabernet Sauvignon occurred in response to water deficit.

MATERIALS AND METHODS

One-year-old *Vitis vinifera* L. cv. Cabernet Sauvignon grafted on 1103Paulsen rootstock (*V. berlandieri* x *V. riparia*) was grown in 18 l high density polyethylene pots using a mixture soil:vermiculite:sand (3:1:1). Plants were cultivated in the greenhouse under natural light conditions, 27/22ºC day/night temperature, 13/11 h light/dark cycle, and relative humidity 78/95% day/night. The experiment was carried out in two uniforms groups of twenty pots each. The first group (control) was daily irrigated in order to maintain the soil water close to the field capacity, while the second group (stressed) has its irrigation suspended during 12 days. All data were subjected to statistical analysis of variance (ANOVA).

Gas exchange measurements were performed on fully expanded leaves with a portable steady-state gas-exchange system (Li-6200, Licor, USA) on four replicates per treatment at 1, 6 and 12 days after water depriving. It was measured and calculated the data of assimilation rate (A), stomatal conductance (gs), transpiration rate (E) and internal CO_2 concentration (Ci).

Respiration was measured as O_2 uptake using a Clark type electrode (Hansatech LD2/3 leaf disc electrode unit). Optimal concentrations of inhibitors as well as the time of incubation, sufficient for obtained maximum inhibition of the respective pathway, were revealed in preliminary experiments by means of titration. Then, to assess the balance between the cytochrome and alternative pathways, the cytochrome *c* pathway was inhibited by 1 mM KCN, whereas the AOX pathway was inhibited by 10 mM salicyldydroxamic acid (SHAM). Residual respiration (V_{res}) was measured in the presence of both the 1 mM KCN and 10 mM SHAM and the AOX capacity (V_{aox}) was estimated by the O_2 uptake measured in the presence of 1 mM KCN after discounting V_{res} (Vanlerberghe et al., 1998; Feng et al., 2008). Both respiratory inhibitors were supplied for 4 h trough the petiole of detached leaves and maintained at a very low light intensity (PPFDs of around 10-20 μmol m^{-2} s^{-1}). For stressed plants, solutions of respiratory inhibitors were supplemented with 500 mM mannitol to maintain the water potential similar to that of undetached stressed leaf tissues (data no shown). Total respiration was measured in leaves without any inhibitor treatment.

Predawn water potential (Ψ_{wPD}) was measured on fully expanded leaves on 1, 3, 6, 10 and 12 days after water depriving in a pressure chamber (Soil Moisture Equipment, USA).

The amount of MDA was measured as described by Dhindsa et al., (1981). Briefly, pre-weighed (0.4 g) fresh leaf sample was ground to a fine powder in trichloroacetic acid 0.1 % added of polivinylpolipirrolidone (PVPP). The homogenate was centrifuged at 4 ºC for 5 min at 20 000 *g*. A 0.5 mL aliquot of the extract was then mixed with the 4.0 mL of TCA 20 % + thiobarbituric acid 0.5 %. The mixture was heated at 95 ºC for 30 min and the reaction was stopped quickly by placing the sample in an ice bath. The absorbance was determined at 532 and 600 nm in a microplate reader Quant (Biotec, USA). After subtracting the non-specific absorbance at 600 nm, the MDA concentration was determined using the extinction coefficient of 155 mM^{-1} cm^{-1}.

Electrolyte leakage (EL) was measured as described by Lutts et al. (1996) with few modifications. Dish leaves was washed with deionized water, placed in tubes with 15 mL of deionized water and incubated for 2 h at 25 ºC. Subsequently, the electrical conductivity of the solution was determined (L_1). Samples were then boiling at ºC for min and the final conductivity (L_2) was measured after equilibration at 25 ºC. The EL was defined as follows: EL (%) = (L1/L2) x 100.

Leaf chlorophyll (Chl) was extracted by soaking 0.1 g leaves in 20 mL dimethylsulphoxide in the dark for 72 h (Hiscox and Israeltem, 1979). Absorbance of extracted Chl was measured at 665 nm (Chla) 649 (Chlb) using a microplate reader μQuant (Biotech, USA) and calculated as described by Lichetenthaler and Wellburn (1983).

RESULTS

Water stress was induced gradually in Cabernet Sauvignon plants by withholding water during 12 days. Drought stressed plants showed a progressive reduction of the predawn leaf water potential (Ψ_{wPD}) ranging from -0,2 in well-watered plants to 1.7 MPa during drought development (Figure 1). Photosynthesis (A_N), stomatal conductance (gs), transpiration (E) and internal concentration of CO_2 (Ci) decreased in response to the water deficit until reach the lowest values of 1.78 μmol m^{-2} s^{-1}, 0,012 mol m^{-2} s^{-1}, 0.55 mmol H_2O m^{-2} s^{-1} and 194.5 ppm, respectively at the 12th day (Figure 2).

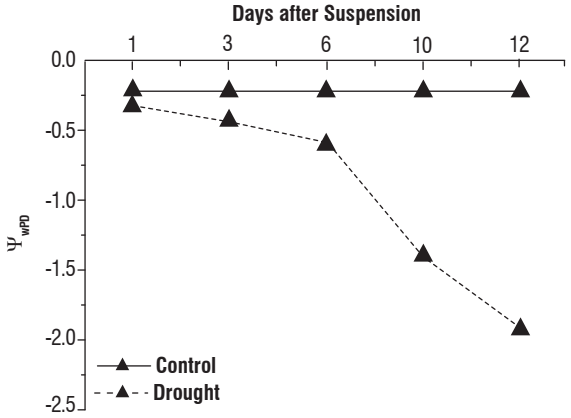

Figure 1. Predawn water potential (Ψ_{wPD}) measured at 1, 3, 6, 10 and 12 days after water depriving in fully expanded leaves of control and drought stressed Cabernet Sauvignon. Values are the means ± SEM of 4 replicates per treatment (error bars are smaller than symbol size).

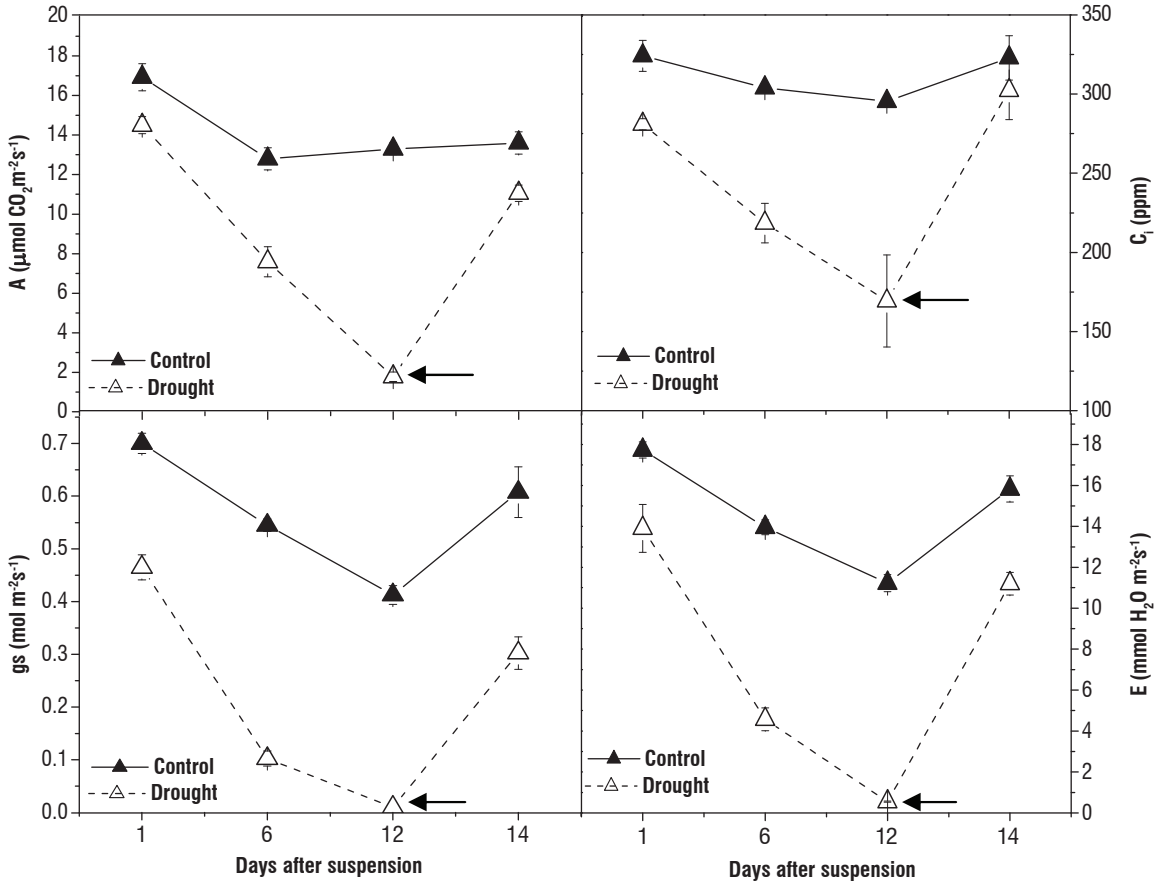

Figure 2. Photosynthetic rate (A_N), internal concentration CO_2 (Ci), stomatal conductance (g_s) and transpiration (E), in leaves of control (▲) and drought (Δ) Cabernet Sauvignon, measured at 1, 6 e 12 days after water depriving. Values are means ± SEM of four replicates per treatment. The arrows showed the re-irrigation of drought stress plants and the measures was taken 48 h after this.

Water use efficiency (WUE) of photosynthesis was estimated by measuring the instantaneous gas-exchange of a leaf as expressed by photosynthesis/transpiration (A_N/E) ratio. Drought stressed plants exhibited WUE 38 and 175 % higher than control at 6 and 12 day after water depriving, respectively. On the other hand, the A_N/C_i ratio a carboxylative efficiency indicative, decreased drastically with progress of stress (Table 1).

The respiratory rate of Cabernet Sauvignon leaves increased around 74 % in response to drought (Figure 3). This respiratory increase was due mostly to an enhancement of the alternative pathway activity as observed in leaves treated with 1 mM KCN. When the residual respiration (0_2 uptake in the presence of KCN + SHAM) is subtracted from the values of Figure 3, it is revealed that the cyanide insensitive oxygen consumption increased four-fold in leaves of stressed plants (Table 2). Conversely, the cytochrome pathway was only slightly activated by the water deficit and its contribution to the total respiratory capacity decreased by three times in relation to the AOX capacity, as revealed by the treatment of the leaves with 10 mM SHAM, an effective AOX inhibitor (Table 2).

Table 1. Water use efficiency (photosynthesis/transpiration rate, A/E) and carboxilative efficiency (photosynthesis/internal CO_2 concentration, A/Ci), recovered at 1, 6 and 12 days after water depriving control and drought plants. The 14th day presents the values recorded 48 hours after re-irrigation of drought plants. Values are means ± SEM of four replicates per treatment.

		Control	Drought
A/E	1	1.02 ± 0.02	0.89 ± 0.03
	6	0.92 ± 0.03	1.27 ± 0.07
(μmol CO_2/mmol H_20)	12	1.2 ± 0.1	3.3 ± 0.4
	14	0.86 ± 0.04	0.99 ± 0.06
A/Ci	1	0.055 ± 0.002	0.047 ± 0.001
	6	0.04 ± 0.002	0.029 ± 0.001
(μmol CO_2/ppm CO_2)	12	0.045 ± 0.001	0.012 ± 0.003
	14	0.042 ± 0.003	0.037 ± 0.002

Table 2. Estimation of the Capacities of Respiration Pathways in Grapevine Leaves. Rates were calculated from data of Figure 3. Residual respiration (O_2 uptake in the presence of 1 mM KCN + 10 mM SHAM was subtracted from all values. Vt is the rate of O_2 uptake in the absence of inhibitors. V_{cyt} is the capacity of the cytochrome pathway, estimated as inhibition in the presence of 10 mM SHAM. V_{aox} is the capacity of the AOX pathway, estimated as inhibition in the presence of 1 mM KCN. All measurements were made in the fully expanded leaf. Values are means ± SEM of three to four replicates.

Treatment	V_t	Respiratory Capacities				
		V_{cyt}	V_{aox}	V_{cyt}/V_{aox}	V_{cyt}/V_t	V_{aox}/V_t
	(μmoles O_2 . g^{-1} DW h^{-1})			(ratio)	(%)	(%)
Control	46.9 ± 4.6	23.1 ± 2.9	15.2 ± 2.1	1.5	49	32
Drought	90.8 ± 5.7	31.3 ± 1.8	60.7 ± 5.3	0.5	34	67

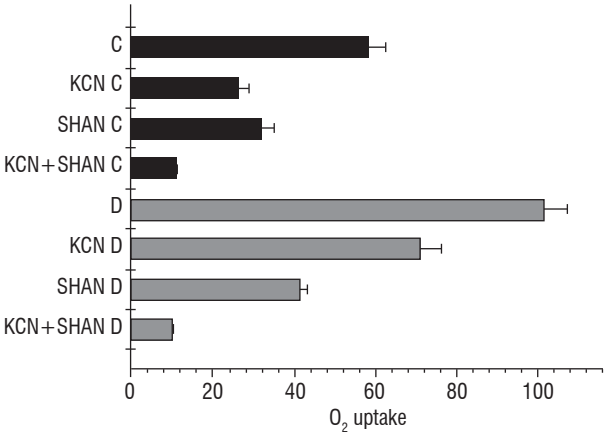

Figure 3. Respiratory activity of Cabernet Sauvignon leaves. Oxygen consumption was monitored in young leaves of plants either irrigated (C) or exposed to drought treatment (D) and following the addition of 1 mM KCN, 10 mM SHAM or both 1 mM KCN + 10 mM SHAM.

DA is one of the end products which is produced as a result of lipid peroxidation damage by free radicals. MDA content was higher in drought-stressed leaves (6.19 μmol g^{-1} FW) than control leaves (2.93 μmol g^{-1} FW) (Table 3). Increased in lipid peroxidation is a possible signal of oxidative damage. Under environmental stresses plant membranes are subject to changes often associated with the increases in permeability and loss of integrity. Therefore, the ability of cell membranes to control the rate of ion movement in and out of cells is used as a test of damage to a great range of tissues. However, no electrolyte leakage was observed in leaves of drought stressed despite the fact that the relatively high lipid peroxidation and thus membrane injury (Table 3).

The $Chlorophyll_{(a+b)}$ amount per unit leaf area was 28 % lower in drought stressed plants, however, the Chl_a/Chl_b ratio was unaltered by stress (Table 3).

Table 3. Malondialdehyde content (MDA), electrolyte leakage (EL), chlorophyll total ($Chl_{(a+b)}$) and chlorophylla/chlorophyllb (Chl_a/Chl_b) ratio in leaves of control and drought-stressed Cabernet Sauvignon plants. Values are means ± SEM of three replicate per treatment.

	MDA (mmol g^{-1} FW)	EL (%)	$Chl_{(a+b)}$	Chl_a/Chl_b
Control	2.9 ± 0.3	3.5 ± 0.5	14.7 ± 0.4	3.3 ± 0.1
Drought	6.2 ± 0.8	4.2 ± 0.6	10.6 ± 1.1	3.4 ± 0.1

DISCUSSION

Water stress has been associated with cell oxidative damages due to enhanced accumulation of ROS, particularly $O_2^{\cdot-}$ and H_2O_2, in chloroplasts, mitochondria, and peroxisomes (Asada, 1999; Pastore et al., 2002; Foyer and Noctor, 2003). Drought-related physiological changes, such as decrease in leaf water content and stomatal closure, result in limited CO_2 availability and channeling of reduced equivalents to the production of active oxygen species rather than CO_2 fixation (Osmond, 1981; Krause and Cornic, 1987). In the present study, stomatal conductance, assimilation rate and internal CO_2 concentration in the leaves of stressed-plants decreased 98 %, 83 %, and 21 % respectively, along with the progress of the water deficit treatment (Figure 3). In this condition, the water use efficiency (A/E) enhanced up to 2.7 fold, while the carboxilative efficiency (A/Ci) decreased 5 fold. However, after 48h of rewatering (at the 14th day) 80 % of A/Ci was recovered (Table 2). These results suggest that photosynthetic machinery of Cabernet Sauvignon is not impaired by water depriving. According to Passioura`s theory of plant water-use behaviour (1982), the Carbenet Sauvignon stressed with high water use efficiency appear to employ a conservative strategy in the use of water. If the photosynthesis decrease is promoted by stomatal closure, an increased water use efficiency is expected. This behaviour reflects a high degree of adaptation of grapevines to drought, in which a variation in Ψ_{wPD} induces a tight control of stomatal closure to stabilize the plant water status. This control will enable winegrapes to growth and even benefits of water deficit regimes, as reported to Cabernet Sauvignon which exhibited increased yield, malic and total acidity, and earlier ripening (Nadal and Arola, 1995).

Physiological adaptations of *V. vinifera* to drought tolerance also involves a tight regulation of the mitochondrial metabolism, as the leaves oxygen consumption markedly increased upon water deficit and this effect was demonstrated to be due to a strong activation of the AOX pathway. It was also observed a striking increase of electron partitioning through the AOX pathway as previously reported for soybean under severe water stress (Ribas-Carbo et al., 2005). However, the soybean AOX enhancement was not higher than 40 %, whereas the AOX capacity of this grapevine was stimulated by up to four times while its contribution in relation to the total respiratory activity enhanced about three times.

It is worth noting that photosynthesis also requires interactions of chloroplasts with the mitochondria to attain optimal rates (Hoefnagel et al., 1998; Padmasree and Raghavendra, 1999; Dutilleul et al., 2003; Noctor et al., 2004; Padmasree et al., 2008; Nunes-Nesi et al., 2008). In this context, it is likely that the stimulation of the AOX pathway observed in grapevines might represent an important response protecting the photosynthetic machinery under conditions of water depriving. Indeed, as photosynthesis decreased under water stress, an excess of reducing power is frequently generated and thus over-reduction of photosynthetic electron chain may result in the formation of ROS that cause oxidative damage as revealed by the reduction (27 %) in total leaf $Chl_{(a+b)}$ (Table 3). The decrease of chlorophyll content is a typical symptom of oxidative stress and may be the result of chlorophyll degradation or synthesis deficiency and with changes of thylakoid structure.

Another evidence of the relevance of oxidative stress to Cabernet leaves was the increased levels (2.7-fold) of MDA content (Table 3). Lipid peroxidation is a natural metabolic process under normal aerobic conditions and is one of the most investigated ROS actions on membrane structure and function (Blokhina et al. 2003; Taylor et al. 2004). It is widely reported that ROS induce peroxidation of membrane lipids leading to membrane damages, often associated with increased permeability and loss of integrity (Kumar and Knowles, 1993; Bolu and Polle, 2004). Nonetheless, the plants of cv. CS preserved the ability of their cell membranes to control the rate of ion movement in and out of cells, since no electrolyte leakage was observed in leaves of stressed-plants.

Survival under drought conditions is closely associated with the plant ability in delaying or preventing the oxidation of cellular components and metabolites (Singh and Rajini, 2004). Under several adverse environmental conditions the AOX is up-regulated (Rizhsky et al., 2002; Bartoli et al., 2005; Yoshida et al., 2007) and the enhancement of the alternative

pathway under drought was previously shown to play a role in limiting the production of ROS (Feng et al. 2008). Actually, it is very likely that a special modulation of the alternative pathway may account, at least in part, for the drought tolerance of *Vitis vinifera* as the AOX seems to be constitutively activated in this species (15.2 μmol O_2 g^{-1} DW h^{-1}), and further increases with drought (60.7 μmol O_2 g^{-1} DW h^{-1}). These values are much higher than that found to wheat (Bartoli et al. 2005) and Arabidopsis (Yoshida et al. 2007).

In conclusion, the present results confirm and expand the notion that *Vitis vinifera* L. cv. Cabernet Sauvignon is able to adapt their photosynthetic process to a reduction in water availability (Iaccono and Sommer, 2000; Guan et al. 2004). The results also reveal that the AOX activation integrates an intrinsic mechanism of this species, which maintains its cellular integrity and functionality under drought stress. Further studies should be directed to understanding the mechanistic details of this AOX control towards developing new biotechnological strategies to improve the stress tolerance, productivity and fruit quality of this important plant species.

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