The First Step from Light to Wine: Photosynthetic Performance and Photoprotection of Grapevine (*Vitis vinifera* L.) Leaves

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ABSTRACT

Grapevine (*Vitis vinifera*) cultivation is successfully established across large areas of the globe whose environmental conditions range from those of the northern temperate zone of the Rhine valley to those of the driest and hottest regions of the Greek islands and the Californian desert. The photosynthetic metabolism follows the C_3 pathway and the responses of the photosynthetic machinery to essential environmental factors are similar to other C_3 plants. The high adaptability of vines to a plethora of different environments is derived from several photosynthetic and associated anatomical and biochemical characteristics of the grapevine leaf. The tight stomatal control coupled with the heterobaric construction of the leaf may offer advantages in water conservation and light utilization and therefore in the photosynthetic performance. Leaves of some cultivars may transiently be anthocyanic or pubescent, characteristics that are believed to be related to the protection against strong visible and ultraviolet radiation, especially during the sensitive stages of initial leaf development. An array of biochemical mechanisms renders the leaves of grapevine highly resistant to photoinactivation and lessens the possibility of photooxidative damage. Since grapevine leaves are the source of the biomolecules which determine the quantity and quality of the fruits, any knowledge on the leaf structure-function relationships and on the leaf-environment interactions is of particular importance to viticulture.

CONTENTS

INTRODUCTION

Grapevine (*Vitis vinifera* L.) is the most widely grown fruit crop in the world since every year 7.3 million hectares of vineyards are harvested globally on all continents excluding Antarctica, yielding approx. 66 million metric tones of grapes (Food and Agriculture Organization of the United Nations 2005). Although more than 80% of the produce is destined to wine making, grapevine also supplies foodstuff and industrial products (Mullins *et al.* 1994) such as jam, grape juice, jelly, resins and grape seed oil. *V. vinifera* is a temperate-climate species which is sensitive to extreme winter temperatures and requires relatively high summer temperatures for the maturation of berries (Mullins *et al.* 1994). For this reason, the majority of the vineyards world-wide are established in Mediterranean type climates. In tropical climates *V. vinifera* behaves as an evergreen plant, producing poorly and tending to be short lived (Winkler *et al.* 1974). There are currently over ten thousand cultivars derived from this single species. The large genetic diversity available and the low chilling requirements for the release of dormancy in buds are considered as the main reasons for the widespread distribution of the grapevine crop (Williams *et al.* 1994). Rootstock genotype affects vine gas exchange, water status, canopy growth and yield (Soar *et al.* 2006).

GENERAL CHARACTERISTICS OF GRAPEVINE LEAVES

The leaf consists of the lamina and the petiole. A pair of

Invited Review

Table 1 Summary of morphological and physiological characteristics related to photosynthetic function of the leaves of *V. vinifera* grown under different light regimes.

	Sun (1500-2000 $\mu mol\ m^{-2}\ s^{-1}$)	Shade (see references for light conditions)	References
Leaf thickness (μm)	120-151	106-131	Ortoidze and Düring 2001[1]
Leaf inclination (degrees)	81.4	15.4	Palliotti and Cartechini 2001[2]
Photosynthetic CO_2 assimilation rate ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	12.7-16.7	8.8-11.4	Ortoidze and Düring 2001
Stomatal conductance ($\mu mol\ H_2O\ m^{-2}\ s^{-1}$)	70-95	45-68	Ortoidze and Düring 2001
Rubisco activity ($\mu mol\ CO_2\ mg^{-1}\ protein\ h^{-1}$)	48.6-52.7	34-37.4	Bertamini and Nedunchezhian 2001[1], 2002[2]
Chlorophyll a/b ratio	2.71-2.8	2,44-2.2	Bertamini and Nedunchezhian 2001[1], 2002[2]
Light saturation point ($\mu mol\ m^{-2}\ s^{-1}$)	806	332	Palliotti and Cartechini 2001[2]
Light compensation point ($\mu mol\ m^{-2}\ s^{-1}$)	60	28	Zufferey *et al.* 2000; Palliotti and Cartechini 2001[2]
Quantum yield	0.034	0.041	Palliotti and Cartechini 2001[2]
Risk of photoinhibition	low	high	Bertamini *et al.* 2004[2]; Ortoidze and Düring 2001[1]

[1]shade conditions: leaves taken from the inner part of the canopy, light intensity 80-120 $\mu mol\ m^{-2}\ s^{-1}$
[2]shade conditions: vines were kept under a black polypropylene cover (40% light transmittance, light intensity 800 $\mu mol\ m^{-2}\ s^{-}$

stipules, ephemeral appendages, protects the young developing leaves (Fahn 1991). The leaf is bifacial (dorsi-ventral), with two types of photosynthetic parenchyma, palisade and spongy, occurring within the mesophyll (Esau 1965). Leaves are hypostomatous since stomata occur only in the abaxial surface of the lamina.

V. vinifera belongs to the functional group of C_3 plants. Thus, the mesophyll cells assimilate CO_2 from the atmosphere through the Calvin cycle, possess photorespiratory activity and show the typical photosynthetic responses to environmental factors similar to other C_3 plants (Williams 1996). Maximum net CO_2 assimilation rates fall in the range between 8 and 13 $\mu mol\ CO_2\ m^{-2}\ s^{-1}$ (Downton *et al.* 1987; Correia *et al.* 1990, see also **Table 1**), although rates of 20 $\mu mol\ CO_2\ m^{-2}\ s^{-1}$ have also been reported (Kriedeman *et al.* 1970; Roper and Williams 1989; Düring 1991). Photosynthetic capacity, measured by a leaf disc oxygen electrode (Nikolopoulos *et al.* 2002) or by a CO_2 analyzer (Düring 1991) at optimal conditions and 5% CO_2 concentration, reaches values of up to 15-20 $\mu mol\ O_2\ m^{-2}\ s^{-1}$. In the absence of stress factors, the CO_2 photorespiratory losses range from 13% to 16% of the net assimilation rate (Düring 1988, 1991). CO_2 compensation point of mature leaves is around 25 μbar (Düring 1991). The estimated water use efficiency of irrigated vines grown in a shaded glasshouse is 3.5-4.7 $\mu mol\ CO_2\ \mu mol\ H_2O^{-1}$ (Düring 1988). Under field conditions the estimated values range between 2 and 5 $\mu mol\ CO_2\ \mu mol\ H_2O^{-1}$ (de la Hera *et al.* 2007).

FACTORS AFFECTING GRAPEVINE LEAF FUNCTIONAL PERFORMANCE

Light

Leaves of field-grown grapevines are exposed to different light intensities during the day according to their position on the canopy. Leaves at the outer part of the canopy receive high light intensities during the day (sun leaves), whereas leaves inside the canopy are generally exposed to lower light intensities (shade leaves). Sun and shade leaves show distinctly different photosynthetic characteristics, as a result of their acclimation to different light intensities. Maximum CO_2 assimilation rates, stomatal conductance, Rubisco activity, light saturation point, light compensation point and chl a/b ratio are higher in sun than in shade leaves (**Table 1**). Moreover, the mean leaf blade inclination and the leaf thickness are higher in sun than in shade leaves (**Table 1**). Palliotti and Cartechini (2001) estimated quantum

yield at 0.034 and 0.041 for sun and shade leaves respectively. Due to the low light intensities under which shade leaves are grown, they show limited capacity for photoprotective responses such as energy dissipation (**Table 1**). The β-carotene and lutein content and the xanthophyll pool sizes of these leaves are generally lower than that of sun leaves (Düring 1998; Ortoidze and Düring 2001), although different data have also been reported (Bertamini and Nedunchenzhian 2004). Mature shade leaves show 35-40% lower values of *in vivo* nitrate reductase activity, soluble proteins, maximum electron transport rate and triose phosphate utilization compared to sun leaves (Bertamini and Nedunchezhian 2001; Bertamini and Nedunchezhian 2002; Schultz 2003b). The above mentioned effects of light regime on photosynthetic metabolism of sun and shade leaves may be modulated by other factors such as temperature, age, type and the stage of the growing season of a leaf (see below).

As mentioned above, photosynthetic CO_2 assimilation of sun-exposed leaves of *V. vinifera* is saturated at light intensities of around 800 $\mu mol\ m^{-2}\ s^{-1}$, considerably less than in full sunlight (around 2000 $\mu mol\ m^{-2}\ s^{-1}$). Therefore, on sunny days in the field, leaves absorb excessive light energy which may cause photooxidative damage to the photosynthetic apparatus. Indeed, under field conditions, photoinhibition of photosynthesis is considered to be a potential threat for the grapevine leaves (Chaves *et al.* 1987; Correia *et al.* 1990). Moreover, the combination of high light intensities with other environmental stress factors, such as severe water stress at midday (Düring 1998), extreme temperatures (Gamon and Pearcy 1990a, 1990b; Hendrickson *et al.* 2004), and high UV doses (Pfündel 2003), may cause severe photoinhibitory damage to PS II (see below; see also Chow *et al.* 2005). Fortunately, excess light energy beyond that used to drive photosynthetic electron flow may be dissipated by a number of protecting biochemical mechanisms. According to Chaumont *et al.* (1995, 1997), the dissipation of excess energy as heat through the xanthophyll cycle accounts for almost all non-photochemical quenching in *V. vinifera* leaves. It is estimated that, under non-stressful conditions, 45-64% of the absorbed light energy is dissipated by this biochemical cycle, whereas under stress, this proportion may rise to 75-92% (Flexas and Medrano 2002; Medrano *et al.* 2002; Hendrickson *et al.* 2004). Moreover, daily fluctuations of the xanthophyll cycle are associated with the degree of photosynthetic depression often observed at noon (Chaves *et al.* 1987; Düring 1991). Consequently, cycle engagement is maximal at noon due to overexcitation of the reaction centers (Düring 1999).

Water availability

Soil water deficits affect both the development of young leaves and the photosynthetic performance of mature leaves. Shoot growth is extremely sensitive to water deficit (Keller 2005) and the reduction of the number of leaves on branches occurs soon after the beginning of soil drying (Lebon *et al.* 2006). This response may be characterized as the first line of defense against extensive water losses and is not related to carbon availability, photosynthetic activity or the content of soluble sugars of the young expanding leaves (Lebon *et al.* 2006). Cell expansion requires cell wall loosening and positive turgor within cells. Young leaves posses higher cell wall elasticity than the mature ones. This enables cells to maintain a positive turgor, enough to allow cell elongation and thus plant growth even under mild water stress conditions (Patakas *et al.* 1997). However, severe water stress may cause complete inhibition of leaf growth (Schultz and Matthews 1993; Lebon *et al.* 2006). The inhibition of leaf expansion under these conditions may be attributed to the decreased cell wall extensibility of the cells (Schultz and Matthews 1993).

Water stress also increases the water use efficiency as well as the rates of photorespiration (Düring 1988; Cifre *et al.* 2005). Under mild drought conditions, stomatal closure appears to be the main cause for the decrease of the photosynthetic activity of the mature grapevine leaves (Flexas *et al.* 1998; Chaves *et al.* 2002; de Souza *et al.* 2005). There are indications that stomatal responses are linked mostly to soil moisture content rather than to leaf water status (Chaves *et al.* 2002; Paranychianakis *et al.* 2004). The tight regulation of stomatal control under mild drought conditions makes stomatal conductance a useful indicator of water stress in grapevine (Cifre *et al.* 2005). Experiments with field-grown vines showed that as summer drought progresses, stomata respond to ABA produced by the water-stressed roots in parallel to the maintenance of the leaf water status under constant levels (Correia *et al.* 1995; Stoll *et al.* 2000). In the long term, stomatal closure is accompanied by down-regulation of photosynthetic machinery in order to acclimate to the low CO_2 availability. Under these conditions, the activity of certain enzymes of the Calvin cycle, the apparent quantum yield are reduced (Correia *et al.* 1990; Chaves *et al.* 2002; Maroco *et al.* 2002; Bertamini *et al.* 2006a) and proline content is increased (Bertamini *et al.* 2006a). Usually, under field conditions water stress does not induce photoinhibition, even when stomatal conductance and photosynthetic rates are reduced to very low levels (Flexas *et al.* 1998; Medrano *et al.* 2002; de Souza *et al.* 2005). It seems that increased electron transport to alternative pathways prevents further down-regulation of photochemical reactions. Under these conditions, increases in thermal energy dissipation (mainly through the xanthophyll cycle) account for up to 90% of total energy dissipation (Medrano *et al.* 2002). High photorespiratory activity under drought stress may also provide additional protection against photoinhibitory damage (Guan *et al.* 2004).

Partial drying of the root system may affect shoot growth and physiological parameters of the leaves. Photosynthetic activity and stomatal conductance are partially reduced in response to experimental treatments where one half of the root system of the vines is subjected to drought, without associated change in shoot water status (Dry and Loveys 1999; Dry *et al.* 2000), although differences may be observed between cultivars (de Souza *et al.* 2005b). Hormonal signals, possibly ABA, synthesized by the roots of the drying zone are probably transported through the transpiration stream to the leaves (Dry and Loveys 1999). This manipulation may cause an increase in water use efficiency of the vines (de Souza *et al.* 2003, 2005a, 2005b; de la Hera *et al.* 2007).

It seems, however that ABA is not responsible for other stomatal responses, such as the diurnal variation in stomatal conductance observed in some cultivars (Correia *et al.* 1995). During the warm days of spring and summer, stomatal aperture decreases progressively after a mid-morning peak (Loveys 1984; Loveys and Düring 1984; Chaves *et al.* 1987; Tenhunen *et al.* 1987; Düring 1991; Patakas 1993; Chaumont *et al.* 1994). This reduction (termed anisohydric behavior) is observed even in well watered plants, but is more intense under water stress conditions (Downton *et al.* 1987; Correia *et al.* 1995; Cuevas *et al.* 2006). However other cultivars behave as isohydric plants that maintain a near constant leaf water potential during the day, irrespective of the soil water status. These differences between cultivars may be attributed to differences in hydraulic architecture mainly of the petiole of the leaves (Schultz 2003a). Stomatal closure may prevent embolism in anisohydric varieties and the signal could be derived from cavitations (Schultz 2003a). These differences between cultivars may be responsible for the observed variation in transpiration efficiency and carbon isotope discrimination among grapevine varieties (Gibberd *et al.* 2001).

Under severe drought, photosynthetic metabolism is progressively impaired. In contrast to the stomatal limitation under mild water stress, this phenomenon is irreversible (Escalona *et al.* 1999; Keller 2005). The depression of photosynthesis is accompanied only by a slight decrease in respiration and thus the carbon balance is disturbed (Keller 2005). Moreover, the combination of severe water stress and high light intensities at midday may cause photoinhibitory damage to PS II (Downton 1987; Chaves *et al.* 2002). Water-stressed leaves of *Vitis californica* exposed to high light intensities exhibit greater reductions of net CO_2 assimilation rates than water-stressed plants exposed to low light intensities (Correia *et al.* 1990; Gamon and Pearcy 1990a).

Salinity

Grapevine is referred either as sensitive or relatively resistant to salinity species, depending on the rootstock. A morphological symptom of vines under osmotic stress in the field is the reduction of leaf area (Ben-Asher *et al.* 2006). Salt treatment (90 mM NaCl) causes recoverable reduction in shoot growth, photosynthetic activity and stomatal conductance (Walker *et al.* 1981; Downton *et al.* 1990). Salinity also causes reduction in the value of carbon isotope discrimination (Δ) and influences N partitioning (Gibberd *et al.* 2003). Vines are able to acclimate to high levels of cellular Cl^- and leaves are able to tolerate Cl^- levels up to 200 mM without sustaining permanent reduction in photosynthetic activity (Walker *et al.* 1981). The decrease in photosynthetic activity has been attributed to stomatal limitation rather than a toxic effect of Na^+ or Cl^- on the photosynthetic machinery (Downton 1977; Walker 1981; Downton *et al.* 1990; Gibbert *et al.* 2003). The accumulation of chloride within leaves at levels of about 350 mM causes necroses in the margins of the leaf blade (Downton and Millhouse 1983).

Reactive oxygen species (ROS) are normally produced as by-products of various metabolic processes and scavenged by biochemical mechanisms in order to prevent damage of sensitive cellular targets. ROS production is increased under stress conditions (Apel and Hirt 2004). In grapevine cell suspensions, salinity stress results in the accumulation of reactive oxygen species (ROS). Moreover, under these conditions, increased proteolytic activity results in the accumulation of ammonium ions at toxic levels and there are indications that produced ROS act as a signal that triggers the expression of anionic glutamate dehydrogenases to form glutamate that is used for proline synthesis. Thus, the increase in the aminating activity of GDH helps grapevine cells to detoxify the high concentration of ammonium ions that is generated by the proteolytic and deaminating activities (Skopelitis *et al.* 2006). Thus, the increase in proline concentration under water stress (Bertamini *et al.* 2006a) may not be directly related to osmoregulation but to the detoxification of ammonium ions.

The relative resistance of grapevine to salt stress is at least partially attributed to osmoregulation. There are indications that under water stress osmotic adjustment is achieved by the accumulation of inorganic ions and not of soluble carbohydrates (Rodrigues *et al.* 1993; Patakas and Noitsakis 2001; Patakas *et al.* 2002).

Temperature

Photosynthetic activity of field-grown grapevines usually shows a quite broad temperature optimum (between 25 and 35°C). Generally it declines at temperatures above 35°C, but positive activities occur even up to 40°C (Kriedemann 1968; Williams *et al.* 1994 and the literature therein; Kadir 2006). The photosynthetic response of grapevine to high temperatures is also strongly genotype-dependent (Kadir 2006). Dark respiration rate, light saturation point and light compensation point respond strongly to temperature, with differences between leaf ages (Zufferey *et al.* 2000). Light saturation point increases with temperature, probably due to the increase of photorespiratory activity (Iacono and Sommer 1996; Zufferey *et al.* 2000). Light compensation point increases strongly with temperature due to differences in dark respiration rates. The effect is more pronounced in young compared to mature leaves (Zufferey *et al.* 2000).

Temperature acclimation of leaves after chilling or heat stress is crucial for tolerance under temperature extremes. Non-acclimated leaves suffer ultrastructural damage in mesophyll cells (Zhang *et al.* 2005). Ca^{2+} ions are involved in the process of acclimation of grapevine mesophyll cells to temperature extremes (Wang *et al.* 2004). Both heat acclimation and exogenous salicylic acid application are also important factors that determine thermotolerance of grapevine leaves. It is probable, therefore, that endogenous salicylic acid play a role in heat acclimation, by inducing the activities of related antioxidant enzymes (Wang and Li 2006).

Chilling temperatures under controlled conditions (between 0 and 15°C) during the night cause photoinhibition, measured by means of F_v/F_m at midday. The ratio of variable (F_v) to maximum (F_m) chlorophyll fluorescence is a parameter derived from the study of fluorescence yield of the PSII reaction center. Decreases in F_v/F_m are associated with decreases in PSII intrinsic photochemical efficiency (Butler and Kitajima 1975). A marked loss of PSII activity, probably due to the loss of D1 and 33kDa proteins of the reaction center, is observed (Balo *et al.* 1991; Bertamini *et al.* 2005a, 2005b, 2006b). However, under field conditions, grapevine leaves remain relatively unaffected by low-temperature-induced photoinactivation of PSII. Under these conditions, the leaves maintain high levels of quantum yield of PSII, despite the reduced energy dissipation by both CO_2 assimilation and photorespiration. It is suggested that the photoprotection is provided mainly through the xanthophyll cycle and to a lesser degree through the Mehler reaction (Flexas *et al.* 1999; Hendrickson *et al.* 2003). A high intrinsic rate of D1 polypeptide repair is also responsible for the resistance of grapevine leaves against photoinactivation (Hendrickson *et al.* 2004). Thus grapevine leaves seem to be more resistant to photoinactivation at high and low temperatures compared to other species (Hendrickson *et al.* 2004; Chow *et al.* 2005).

Leaf age and leaf position on the canopy

Maximum rates of photosynthesis in grapevine leaves are observed 30-40 days after unfolding (just before or at full expansion) and thereafter a gradual decrease occurs (Kriedemann *et al.* 1970; Intrieri *et al.* 1992; Bertamini and Nedunchezhian 2003a). The low photosynthetic activity of the young leaves may be due to immaturity of the photosynthetic machinery (Bertamini and Nedunchezhian 2003a, 2003b), high resistance to CO_2 diffusion due to the absence of intercellular spaces (Kriedeman *et al.* 1970) or the occurrence of non functional stomata. Young leaves show

higher values of the light compensation point, higher proportion of xanthophyll cycle pigments, but lower concentration of total chlorophylls per unit area than the mature ones (Chaves 1981; Bertamini *et al.* 2003b). Young leaves retain most of the carbon they assimilate before they reach ca. 50% of their final size while larger leaves begin to export carbohydrates (Williams 1996). Senescent leaves show a reduction in the overall photosynthetic rates due to marked losses of both PSII and Rubisco activities (Bertamini and Nedunchezhian 2003a). According to Petrie *et al.* (2000), the decline in the photosynthetic rate of mature leaves may be caused by an increase in the source to sink ratio (as measured by the leaf area to fruit weight ratio) due to progressive leaf emergence. Remobilization of nitrogen (either in inorganic or organic form) occurring in shaded or senescing leaves to other parts of the canopy (Keller 2005) may also affect photosynthetic rates.

Leaf position on the canopy also affects photosynthetic rates. Middle leaves are the most functional; however on a whole-leaf basis the basal, larger leaves show the higher contribution (Intrieri *et al.* 1992). Other positional effects such as leaf orientation and mutual shading may determine the photosynthetic performance of individual leaves (Williams *et al.* 1994).

Leaf orientation and position on the canopy are important determinants of leaf temperature. Differences in microclimate between canopy sides resulting in suboptimal leaf temperatures may be responsible for limited carbon gain and subsequent restricted shoot growth rates (Hendrickson *et al.* 2004). Horizontally restrained leaves are exposed to high heat load during summer under field conditions and this may cause severe, long-term damage to one or more of the partial processes of photosynthesis (Gamon and Pearcy 1989). Heat load may be a significant stress factor when stomata are closed. Leaf movements may protect the photosynthetic components during summer extremes. The decrease of the angle between leaf blade and petiole reduces the light absorption and thus the overload of absorbed energy under prolonged water stress or nitrogen limitation (Keller 2005).

Wind

Wind affects stomata behavior of grapevine leaves by reducing stomatal conductance and therefore transpiration rates (Kobriger *et al.* 1984; Campbell-Clause 1998). However, leaf water potential is not affected by wind treatment (Kobriger *et al.* 1984). For moderate wind speed, stomatal conductance is brought back to normal levels within one day after wind break, whereas for high wind speed the effect on stomatal conductance continues for longer periods. No effect is observed on stomatal conductance under moderate wind speed when vines are acclimated under low speed wind (Kobriger *et al.* 1984). In the long-term wind may develop asymmetrical canopies due to wind-induced morphogenesis and thus wind may affect light interception from leaves, photosynthetic activity and fruit development (Tarara *et al.* 2005).

Mineral nutrition

Among all mineral nutrients, nitrogen is the most important for grapevines and may easily limit growth (Keller 2005). A linear relationship between percent N content and net CO_2 assimilation rate expressed either on area or dry weight basis exists (Williams and Smith 1985). Nitrogen deficiency negatively affects photosynthesis by reducing the activity of photosynthetic enzymes, including Rubisco (Chen and Cheng 2003). A reduction in chlorophyll content and activation of thermal energy-dissipation and antioxidant systems to protect from photo-oxidative damage under high light intensities, are also observed (Chen and Cheng 2003; Keller 2005). Under N limiting conditions, growth processes are suppressed more than photosynthesis and this leads to the accumulation of carbohydrates in leaves and

synthesis of carbon-based secondary metabolites, such as anthocyanins, which are accumulated in the petioles and the leaf veins (Keller 2005; Grechi *et al.* 2007). Similar symptoms can also be observed under phosphate deficiency. P limitation can also cause a reduction in stomatal conductance and thus a restriction in cell expansion due to water shortage (Keller 2005).

Iron supply also affects the photosynthetic performance of *Vitis* leaves. Fe limitation causes significant reduction in the activities of certain enzymes of Calvin cycle, including Rubisco, in the PSII activity and hence in the CO_2 assimilation rates (Bertamini *et al.* 2002b; Chen *et al.* 2004; Bertamini and Nedunchezhian 2005c). As a result, Fe-deficient leaves have lower concentrations of non-structural carbohydrates (Chen *et al.* 2004). Under these conditions, photoprotective mechanisms such as the xanthophyll cycle and the ascorbate-glutathione antioxidant system are enhanced to cope with the excess light absorbed (Smith and Cheng 2005).

Fruit load

Net photosynthesis is positively correlated with fruit load (Edson *et al.* 1995; Petrie *et al.* 2000). On the other hand, grapevine leaves are acclimated to fruit loss. Fruit removal does not cause any noticeable changes in photosynthetic function. Under these conditions the carbon budget of the leaves remains unaffected with the synthesis and contents of starch and sucrose remaining rather stable (Chaumont et al 1994). It seems that although the fruits are the major sink for the assimilated carbon, other carbon-requiring processes or carbon stores are activated to balance the carbon flow.

STRUCTURE-FUNCTION RELATIONSHIPS AND STRESS TOLERANCE

The surface of leaves constitutes the first line of defense against external biotic or abiotic stresses. The epidermis and the cuticle play an important role in protecting the mesophyll against water and carbon losses allowing only small amounts of CO_2 and water vapor to pass through. The cuticle shows a CO_2 conductance of only 5.7% that of water vapors (Boyer *et al.* 1997). Therefore, gas exchange is essentially absent in leaves with tightly closed stomata (Boyer *et al.* 1997). The cuticle also contributes to protecting mesophyll tissues against biotic attacks and high doses of UV radiation.

The adaxial epidermis of grapevine leaves contain considerable amounts of phenolic compounds located mainly in the vacuoles of the epidermal cells (Kolb and Pfündel 2005). Synthesis of hydroxycinnamic acids is stimulated by high visible light intensities and flavonoid production is specifically enhanced by UV radiation (Kolb *et al.* 2001). These substances absorb strongly in the UV region and offer considerable protection against the penetration of the harmful UV-B radiation into the mesophyll. Thus, the epi-
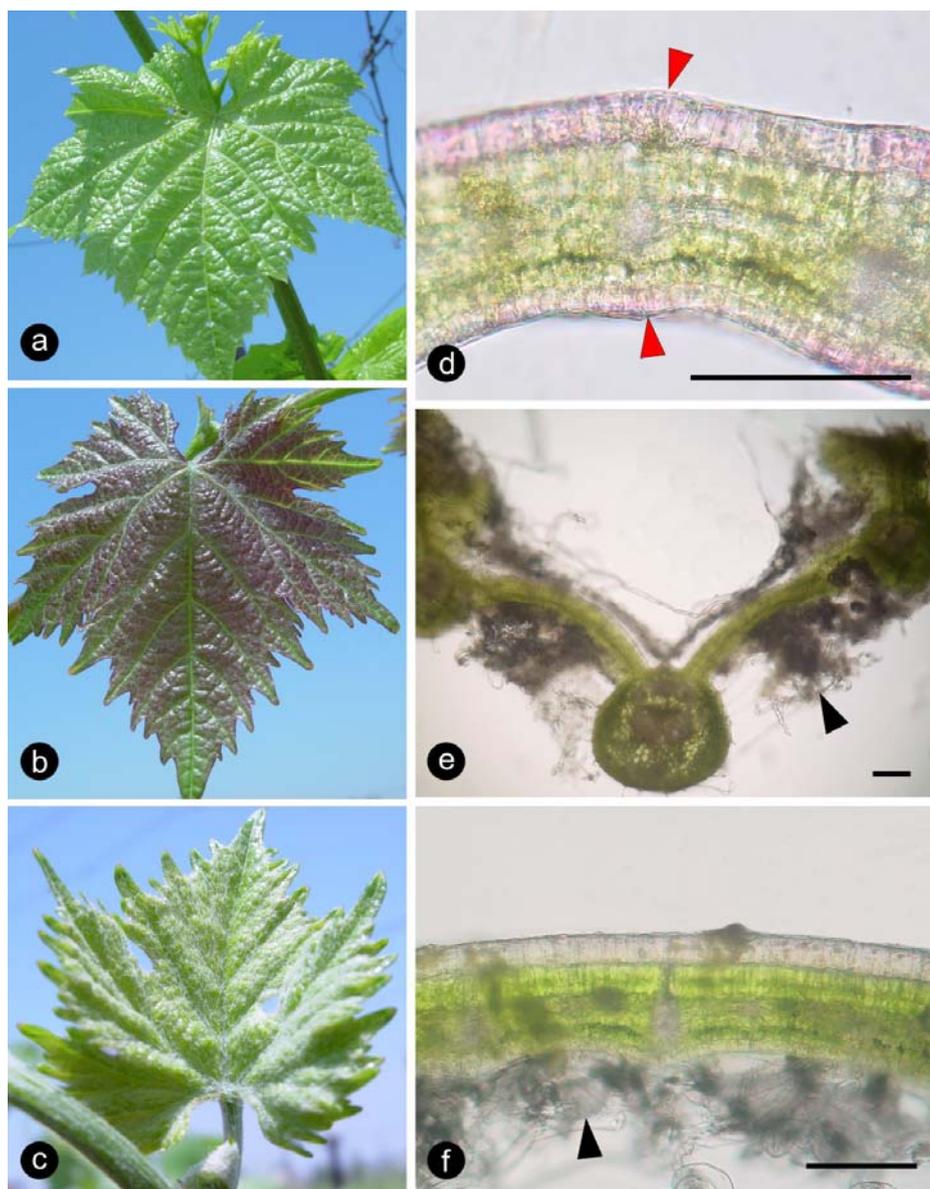

Fig. 1 Young leaves of *Vitis vinifera* cv. 'Soultanina' 35 days after emergence (DAE). (a), cv. 'Siriki', 35 DAE (**b**) and cv. 'Athiri' 21 DAE (**c**). **d-f**. Light micrographs of hand-cut cross sections of young leaves of *Vitis vinifera*. (**d**) developing leaf of cv. 'Siriki' at 28 DAE showing anthocyanic pigmentation of abaxial and adaxial epidermal cells (red arrowheads). (**e, f**) progression of pubescence in developing leaves of cv. 'Athiri'. At very early stages of leaf expansion (14 DAE), hairs appear in both adaxial and abaxial surfaces (**e**). At 35 DAE, hairs remain only at the abaxial leaf surface (**f**; black arrowheads). Scale bar 100 μm. Micrographs taken by Klouvatou, A. (see also Liakopoulos *et al.* 2006).

dermis behaves as a selective optical filter that allows the penetration of visible radiation, but prevents the penetration of UV-B and part of UV-A radiation (Karabourniotis *et al.* 1999).

Morphological characteristics such as the outline, size, color, contour, dentation and venation pattern as well as the pubescence of the leaves are useful characters for cultivar description and identification. Some of these characters affect the optical properties of the leaves and thus the photosynthetic performance of a particular cultivar. *V. vinifera* cultivars offer a model system for testing how epidermal appendages and/or epidermal pigmentation affect the optical properties of leaves and thus the photosynthetic performance of the lamina.

In several cultivars, pubescence on the surface and anthocyanins in the epidermis are particularly abundant during the early developmental stages of the leaves (**Fig. 1**). Transient pubescence or pigmentation is considered as a defensive character against photodamage in developing leaves, as these are more susceptible to photoinhibition compared to the mature ones, due to their immature photosynthetic apparatus (Jiang *et al.* 2006; Liakopoulos *et al.* 2006). The trichome layer behaves as a light screen that reduces the penetration of both UV and visible light into the mesophyll (Karabourniotis *et al.* 1999). Compared to glabrous-green leaves, both anthocyanic and pubescent leaves of the particular *V. vinifera* cultivars show greater dark-adapted PSII photochemical efficiency, net photosynthetic rates and considerably smaller ratio of xanthophylls cycle components and mid-day de-epoxidation state of the xanthophyll cycle. These differences were more evident in pubescent leaves, indicating that trichomes are more effective in protecting mesophyll from light stress than a pigmented epidermis (Liakopoulos *et al.* 2006).

The repeated branching of the veins gives the characteristic palmate venation of the leaf. *V. vinifera* leaves are heterobaric: at least the main veins consist of the vascular bundle surrounded by a sheath which creates extensions (bundle sheath extensions, BSEs) to the epidermis on both sides of the leaf forming transparent regions in the leaf blade. Thus, the mesophyll of grapevine leaves is separated into many small compartments termed 'aeroles' or 'BSE compartments' (Terashima 1992). The occurrence of BSEs in *V. vinifera* leaves has two consequences: A) each aerole behaves as an autonomous photosynthetic compartment that closes or opens its stomata according to environmental (light intensity, water availability, salinity, atmospheric humidity) or internal (ABA) signals (Downton *et al.* 1988a, 1988b, 1990; Düring 1992; Düring and Loveys 1996; Düring and Stoll 1996a, 1996b). Thus, under stress conditions, some aeroles have open stomata while others have not. This non-uniform stomatal closure pattern (stomatal patchiness) causes heterogenous gas exchange and a reduction in photosynthetic activity on a whole leaf basis. This allows a more tight control of water losses and may be beneficial under stress conditions. B) The pigment-free bundle sheath extensions of *V. vinifera* leaf create transparent ribs which surround the photosynthetic parenchyma cells. These structures behave as 'windows' transferring visible light within the internal layers of photosynthesizing mesophyll cells (Karabourniotis *et al.* 2000). The light environment in the interior of each aerole is enriched and the photosynthetic performance is increased accordingly (Nikolopoulos *et al.* 2002).

The particular mesophyll anatomy of the leaves of each cultivar may also affect the photosynthetic performance (Patakas *et al.* 2003a). Patakas *et al.* (2003b) proposed that differences in parameters such as the fraction of mesophyll volume represented by the intercellular spaces and the surface area of mesophyll cells exposed to intercellular airspaces may be responsible for the observed differences in photosynthetic rates between different cultivars.

Calcium oxalate crystals, in the form of raphide bundles, occur in specialized cells (idioblasts) within mesophyll of grapevine leaves (Fabbri *et al.* 1992; Arnott and Webb 2000; Jauregui-Zuniga *et al.* 2003; de Bolt *et al.* 2004). Raphides develop within an organic matrix which provides control for crystal formation. The matrix consists of two structural phases, membrane chambers enclosing developing crystals and a water-soluble phase (containing an unusual polymer with glucuronic acid linkages and inorganic ions, mainly calcium and potassium) surrounding the crystal chambers. Both crystal chambers and matrix contain proteins that promote crystal nucleation (Webb *et al.* 1995). It is believed that raphides are cellular sites of calcium sequestration and that they take part in the defense against herbivores.

CONCLUDING REMARKS

Recent advances in plant science have revealed many aspects of the ability of *V. vinifera* to adapt and/or acclimate across different environments. Recent work has identified three CBF genes as well as two dehydrin genes, related to freezing and drought tolerance, that are expressed as a response to stimulants related to tissue dehydration, namely cold, salinity, drought or ABA treatment (Xiao and Nassuth 2006; Xiao *et al.* 2006). More information at the molecular level on responses related to stress tolerance is urgently needed to assist in producing highly tolerant grape cultivars. At the physiological level, several aspects of the adaptablity of grapevine to various stressful regimes are primarily ascribed to the efficiency of the photosynthetic machinery and the strategic water management of the plant. Grapevine cultivars show markedly different physiological and morphological characteristics which will be evaluated and employed to design improved plants in the near future. Research projects focusing on the correlation between structural and functional aspects, especially under field conditions, will promote the improvement of the grapevine crop yield.

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