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Gas exchange and water relations of three *Vitis vinifera* L. cultivars growing under Mediterranean climate

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Abstract

Optical characteristics, contents of photosynthetic pigments, total soluble sugars, and starch, rates of gas exchange, chlorophyll (Chl) *a* fluorescence, and leaf water relations were analysed in three *Vitis vinifera* L. cultivars, Tinto Cão (TC), Touriga Nacional (TN), and Tinta Roriz (TR), grown in Mediterranean climate. Chl content was significantly lower in TC than in TN and TR leaves, while the Chl *a/b* ratio was higher. TR had the lowest net photosynthetic rate, stomatal conductance, and contents of soluble sugars and starch than TN and TC. In spite of low Chl content, TC showed the lowest photon absorbance and the highest photochemical efficiency of photosystem 2. TC had the lowest predawn and midday leaf water potential. The capability for osmotic adjustment was similar among cultivars and the calculated modulus of elasticity was higher in TC leaves. The typical lighter green leaves of TC seemed to be an adaptive strategy to high irradiance and air temperature associated to water stress.

Additional key words: carotenoids; chlorophyll; internal CO_2 concentration; leaf water potential; net photosynthetic rate; optical characteristics; stomatal conductance; starch and sugars; transpiration rate; water use efficiency.

Introduction

Grapevine cultivars differ by anatomical, physiological, and biochemical characters (Chaves *et al.* 1987, Chaumont *et al.* 1997, Flexas *et al.* 2001) and by colour of leaves. Adaptations to sunny habitats involve genetically fixed modifications of key constituents of the photosynthetic apparatus (Chaves *et al.* 1987). On the greenish-yellow colour leaves, the lower content of photosynthetic pigments decreases leaf absorptance (Schultz 1996) avoiding damage to the photosynthetic apparatus in grapevines exposed to high irradiance and air temperature associated to water stress (Chaumont *et al.* 1997, Flexas *et al.* 2002).

Gas exchange and water relations of three grapevine cultivars widely spread over the Douro region (Northeast Portugal), characterized by different leaf green tonality and cultivated under typical Mediterranean field conditions, were investigated.

Materials and methods

Plants and growth: The research was carried out at the experimental farm “Santa Bárbara” of the Agriculture Extension of Trás-os-Montes e Alto Douro (41°10'N, 7°33'W, 100 m above mean sea level) in the Upper Corgo sub-region of the Douro Wine Region (Northern Portugal) during the years 2002–2004. The area is characterized by a total annual rainfall of 649 mm (data for the period 1961–1990) of which 22 % is from May to

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Abbreviations: Car – carotenoids; Chl – chlorophyll; C_i – internal CO_2 concentration; DM/TM – dry mass/saturated mass; E – transpiration rate; F_0, F_v, F_m – minimum, variable, and maximum fluorescence, respectively; F_v/F_m – photochemical efficiency of photosystem 2 of dark-adapted leaves; g_s – stomatal conductance; P_N – net photosynthetic rate; P_N/g_s – intrinsic water use efficiency; PPFD – photosynthetic photon flux density; PS – photosystem; R:FR – red/far-red ratio (660/730 nm); RWC^0 – relative water content at zero turgor; SS – soluble sugars; ε_{max} – maximum bulk modulus of elasticity; Ψ_π – osmotic potential at full turgor; Ψ_{PD}, Ψ_{MD} – leaf water potential at predawn and midday, respectively.

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September, a mean air temperature of 15.6 °C, a mean minimum air temperature of 9.4 °C, and a mean maximum air temperature of 21.8 ºC (database of Portuguese Meteorological Institute). The vineyard is located on a steep hill on a schistous soil type.

Three *Vitis vinifera* L. Portuguese cultivars were studied: Touriga Nacional (TN), Tinta Roriz (TR, synonymy Aragonês or Tempranillo), and Tinto Cão (TC); this last is characterised by lighter green leaves. At the beginning of the experiment, 20 uniform 12-year-old plants per cultivar were grafted onto R99 (*V. berlandieri*× *V. rupestris*) rootstocks. Plants were spaced at 2.0×1.2 m, trained as bilateral cordons (pruned with 12 buds), and managed without irrigation. All measurements were performed on a typical summer day and on fully expanded sun leaves (40–60 d-old) located in the middle of the shoots.

Photosynthetic pigments, soluble sugar and starch contents were analysed in leaf discs from six leaves per cultivar. They were frozen in liquid N_2 and stored at –80 °C. Chlorophyll (Chl) *a* and *b* and total carotenoid (Car) contents were measured spectrophotometrically in leaf extracts by 80 % acetone (Šesták *et al.* 1971). Car were determined using the Lichtenthaler (1987) equations. Also, Chl contents per leaf area were estimated non-destructively by a *SPAD-502* device (*Minolta*, Osaka, Japan). The *SPAD-502* records leaf transmittance of induced light beams in two wavelengths, *i.e.* 650 and 750 nm, the output value can be used as a measure of leaf greenness, and SPAD units are in relationship with extractable Chl and nitrogen contents for many crops (Daughtry *et al.* 2000). SPAD readings were carried out in the field in the same leaf samples utilized for the other measurements.

Soluble sugars (SS) were extracted by heating leaf discs in 80 % ethanol according to Irigoyen *et al.* (1992) and analysed by the reaction of 0.1 cm^3 of the alcoholic extract with 3 cm^3 fresh anthrone placed in a boiling water bath for 10 min. After cooling, the absorbance at 625 nm was determined. After the extraction of soluble fractions, the solid fractions were used for starch analysis. Starch was extracted with 30 % perchloric acid according to Osaki *et al.* (1991), and its content was determined by the anthrone method as described above. Glucose was used as standard for both soluble sugar and starch.

Optical characteristics of leaves: The ratio of quanta (R:FR ratio) transmitted and reflected by leaves was determined in the field under clear sky at midday using a 660/730 nm sensor (*Skye Instruments*, Wales, GB). Transmitted radiation was measured normal to the plane and immediately under the leaf, positioned with its surface perpendicular to the sun. Reflected radiation was measured 1 cm above the same leaf by placing the sensor at an angle of 45° from the perpendicular, according to Schultz (1996).

Gas exchange and Chl *a* fluorescence: Gas exchange parameters were determined with a portable IRGA (*ADC-LCA-3*, *Analytical Development*, Hoddesdon, England), operating in the open mode and a leaf chamber clip (*ADC-PLC-B*) at morning (09:00–10:30), at midday (14:00–15:30), and in the afternoon (16:30–18:00). The photosynthetic photon flux density (PPFD) incident on the leaves was always higher than 1 500 $\mu mol\ m^{-2}\ s^{-1}$, which is above photosynthesis saturation in the considered species (Flexas *et al.* 2002). Net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), and internal CO_2 concentration (C_i) were calculated using the equations of Caemmerer and Farquhar (1981). The P_N/g_s ratio was used as intrinsic water use efficiency, according to Iacono *et al.* (1998).

Chl *a* fluorescence parameters: photochemical efficiency of photosystem 2 (PS2) of dark-adapted leaves (F_v/F_m), minimum (F_0), variable (F_v), and maximum (F_m) fluorescence were measured on the same leaves used for gas exchange measurements by a portable fluorometer (Plant Stress Meter, *BioMonitor SCI AB*, Umeå, Sweden) as described by Öquist and Wass (1988). Before measurements, leaves were adapted to dark for 30 to 45 min, using a clamp cuvette.

Plant water relations: Leaf water potential (Ψ) was determined with a pressure chamber (*ELE International*, England), according to Scholander *et al.* (1965). Measurements were performed on six fully expand leaves at predawn (Ψ_{PD}) and midday (Ψ_{MD}) just after gas exchange measurements. Leaf samples were conserved in a plastic bag immediately after excision.

In order to compare the tissue water relationships and osmotic adjustment of TN, TR, and TC cultivars during summer, pressure-volume (PV) curves were done on six individual fully hydrated leaves per cultivar, using the pressure chamber technique (Tyree and Hammel 1972) according to Rodrigues *et al.* (1993). Leaves were collected early in the morning and brought back to the laboratory in containers partially filled with distilled water so that only the ends of the petioles were submerged; the petioles were then re-cut under water to remove any vapour gap in the xylem. Leaves were covered with a plastic bag and left for 2 h to reach full saturation, at room temperature and low irradiance. Water potential isotherms by a progressive loss of symplastic water were determined. At periodic intervals, samples were weighted and immediately water potential was evaluated using a pressure chamber (internally covered with moist paper to reduce transpiration during measurement). Leaves were dried at constant temperature of 20 ºC and the drying period in each curve was about 6–8 h. Pressure-volume curves were drawn using a type II transformation (Tyree and Ritcher 1982). Osmotic potential at full turgor (Ψ_π), relative water content at zero turgor (RWC^0), and maximum bulk modulus of elasticity (ε_{max}) were measured. Leaves used for the P-V curve analysis were further used to measure dry mass/saturated mass ratio (DM/TM).

Statistical analyses: Data were submitted to analysis of variance and averages were separated with the Duncan's multiple range test at 5 % level.

Results

During a severe drought period in the year 2003, the photosynthetic pigment contents were significantly lower in TC than in TN and TR (Table 1). SPAD readings were 44 and 22 % higher in TR and TN leaves than in TC leaves, respectively (Fig. 1). Chl *a/b* ratio was higher in TC leaves but Chl($a+b$)/Car ratio was not significantly different among cultivars (Table 1). TC leaves were characterized by higher R:FR transmittance and reflectance than TN and TR and, consequently, by lower absorptance (Fig. 1).

The highest P_N and g_s values were found in TN in all periods of the day (Fig. 2). In the midday and afternoon periods (high PPFD, vapour pressure deficit around 5 kPa), TR leaves exhibited the lowest P_N and g_s and the highest C_i.

SS content was significantly higher in TN and TC leaves than in TR ones (Table 1). However, the starch content did not differ significantly among cultivars (Table 1). Similarly, no significant differences were observed in the carbon assimilate partitioning between SS and starch (data not shown).

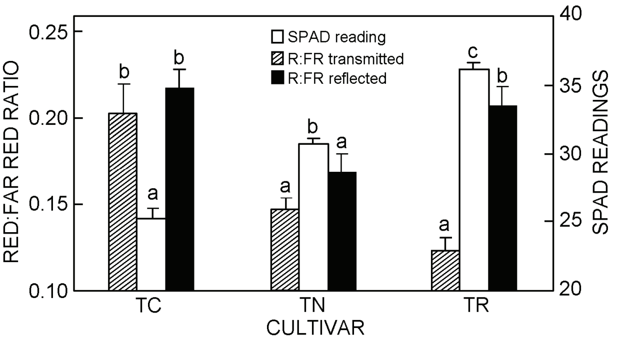

Fig 1. Red/Far Red ratio transmitted and reflected by leaves, and SPAD readings of the considered grapevines cultivars (Tinto Cão – TC; Touriga Nacional – TN; Tinta Roriz – TR) measured in 2003. *Each column* is the average of 6 leaves and *vertical bars* represents the S.E. Within each parameter column headed by the same letter do not differ significantly, according to Duncan's multiple range test at 5 % level.

Table 1. Total contents of chlorophyll (Chl) and carotenoids (Car) [mg m^{-2}], Chl *a/b* and Chl/Car ratios, and contents of soluble sugars (SS) and starch [mg m^{-2}] of Tinto Cão (TC), Touriga Nacional (TN), and Tinta Roriz (TR) cultivars measured in 2003. Means ± S.E. (*n* = 6) followed by the same letter of each parameter do not differ significantly, according to Duncan's multiple range test at 5 % level.

Cultivar	Chl	Car	Chl *a/b*	Chl/Car	SS	Starch
TC	0.208±0.010 a	0.058±0.002 a	3.04±0.02 b	3.60±0.08 a	5.55±0.05 b	2.87±0.17 a
TN	0.313±0.027 b	0.083±0.006 b	2.81±0.06 a	3.76±0.08 a	5.83±0.26 b	2.80±0.22 a
TR	0.409±0.032 c	0.113±0.008 c	2.81±0.04 a	3.61±0.03 a	4.76±0.18 a	2.36±0.17 a

Table 2. Predawn (Ψ_{PD}) and midday (Ψ_{MD}) leaf water potentials and osmotic potential at full hydration (Ψ_π) [MPa], relative water content at turgor loss point (RWC^0) [%], dry mass/satured mass ratio (DM/TM) [kg kg^{-1}], and maximum bulk modulus of elasticity (ε_{max}) [MPa] of Tinto Cão (TC), Touriga Nacional (TN), and Tinta Roriz (TR) cultivars measured in 2003. Means ± S.E. (*n* = 6) followed by the same letter within parameter do not differ significantly, according to Duncan's multiple range test at 5 % level.

Cultivar	Ψ_{PD}	Ψ_{MD}	Ψ_π	RWC^0	DM/TM	ε_{max}
TC	–0.92±0.02 a	–1.71±0.04 a	–1.11±0.04 a	93.6±0.8 a	0.31±0.01 a	10.3±1.5 b
TN	–0.57±0.04 c	–1.36±0.01 c	–1.07±0.07 a	91.3±0.7 a	0.30±0.01 a	5.9±0.6 a
TR	–0.72±0.04 b	–1.55±0.02 b	–1.10±0.09 a	92.3±0.6 a	0.30±0.01 a	7.2±0.7 ab

The lower Chl content of TC leaves did not cause a decrease in PS2 photochemical efficiency (Fig. 3). TC leaves presented slightly high dark-adapted F_v/F_m ratio and high values of F_0, F_m, and F_v in early morning and at midday (*i.e.* at low and high PPFDs, respectively).

Leaf water potentials and parameters of bulk leaf water relations are summarized in Table 2. Ψ_{PD} and Ψ_{MD} differed significantly between cultivars, being higher for TN than TR and TC. No significant differences were observed on Ψ_π, RWC^0, and DM/TM. Nevertheless, TC leaves exhibited the highest ε_{max} (75 and 43 % higher than the TN and TR leaves, respectively).

Discussion

TC leaves developed in typically summer conditions, characterized by drought, high temperatures, and sunny days, showed lowest contents of photosynthetic pigments (Table 1), which gave them lighter green colour. This lower value may indicate poorer leaf nitrogen content, since the majority of leaf N is contained in Chl molecules (Netto *et al.* 2005). On the other hand, in spite of their lower Chl and Car contents, TC leaves showed a greater Chl *a/b* ratio (Table 1), which indicates a preferential lower concentration of Chl *b* content and therefore of antenna light-harvesting complexes. This pattern may be an important mechanism for avoiding high-irradiance damage to the photosynthetic apparatus and suggests a better acclimation of the photosynthetic system to sun-exposed habitats (see review by Chaves *et al.* 2003). Since Chl/Car ratio was not significantly different among cultivars (Table 1), the lower Chl (*a*+*b*) of TC leaves represented an efficient strategy for sun acclimation and not a photo-oxidative or senescence effect (Yoo *et al.* 2003). According to Hendry and Price (1993), Chl tends to be photo-oxidized at high irradiance and, because Car can prevent Chl degradation, the relationship between Chl and Car may be used as a potential indicator of photo-oxidative damages caused by strong irradiation. Differences in Chl content among cultivars confer different optical characteristics (Fig. 1). Leaves with low Chl contents had the highest reflectance and transmittance in the visible and lowest reflectance and transmittance in the near-infrared, in accordance with Daughtry *et al.* (2000).

Although having the highest photosynthetic pigment contents, TR leaves exhibited always the lowest P_N and g_s, and the highest C_i at midday and afternoon periods (Fig. 2). Despite the uncertainty of C_i estimated from gas exchange measurements (Terashima 1992), this pattern of response means that, for beyond the higher stomatal adjustment to avoid excessive water loss under water stress, the non-stomatal limitations as a result of biochemical changes also contributed to the highest reduction of P_N (Schultz *et al.* 1996, Medrano *et al.* 2002, Moutinho-Pereira *et al.* 2004). TC leaves had intermediate P_N and g_s values mainly at midday and afternoon (Fig. 2). Nevertheless, because C_i and P_N/g_s values did not differ significantly between TN and TC in these diurnal periods, the P_N decrease was mostly attributed to stomatal closure. Considering that TR and TN leaves had higher Chl (*a*+*b*) but lower Chl *a/b* than TC leaves (Table 1), these results suggest a high efficiency of photon capturing system of TC leaves.

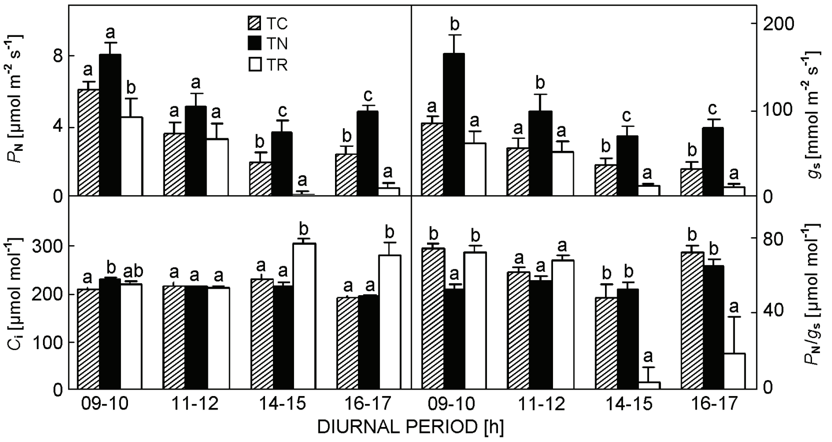

Fig. 2. Diurnal changes in net photosynthetic rate (P_N), stomatal conductance (g_s), internal CO_2 concentration (C_i), and intrinsic water use efficiency (P_N/g_s), of Tinto Cão (TC), Touriga Nacional (TN), and Tinta Roriz (TR) cultivars measured in 2003. *Each column* is the average of measurements on 6 different leaves and *vertical bars* represent the S.E. Within each diurnal period columns headed by the same letter do not differ significantly, according to Duncan's multiple range test at 5 % level.

Probably as a consequence of higher P_N, TN and TC leaves showed higher SS contents than TR leaves, but we did not find differences neither in starch content nor in carbon assimilate partitioning between SS and starch. Under summer stress, many species or cultivars can alter this relationship, resulting in an increase in SS content with the objective of maintenance of metabolic activity, and therefore growth and productivity (Chaves 1991, Bacelar *et al.* 2006). Azcón-Bieto (1983) reported that a high SS content can result in a bad photosynthetic regulation. However, some studies on grapevines under Mediterranean conditions have shown that this possibility is not easily predictable (Chaves 1986, Osório 1994), reinforcing our results. As highlighted by Paul and Driscoll (1997), the apparent sugar repression of the photosynthetic activity depend more crucially on the hexose : amino acid balance, rather than on the saccharide status alone.

The pattern of changes in Chl *a* fluorescence of TC leaves (Fig. 3) suggests a lower proportion of the absorbed photons used in photochemistry than in TN and TR leaves, not only due to lower absorptance, but also due to higher relative amount of photons dissipated thermally by the PS2 antenna (Björkman and Demmig 1987, Demmig-Adams *et al.* 1996). Similar conclusions have been reported in moderate iron-deficient leaves, the most characteristic symptom of which is their greenish-yellow colour (Morales *et al.* 2000). According to these authors, only extremely Fe-deficient leaves showed sustained decreases in PS2 photochemical efficiency and a significant reduction of photosynthetic capacity.

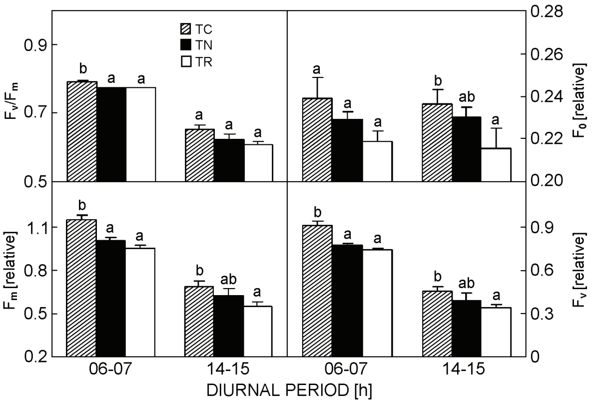

Fig. 3. Photochemical efficiency of photosystem 2 of dark-adapted leaves (F_v/F_m), minimal fluorescence (F_0), maximal fluorescence (F_m), and variable fluorescence (F_v) of Tinto Cão (TC), Touriga Nacional (TN), and Tinta Roriz (TR) cultivars measured at predawn (06–07 h) and midday (14–15 h) diurnal periods measured in 2003. *Each column* is the average of measurements on 6 different leaves and *vertical bars* represent the S.E. The same letter in each parameter/diurnal period shows no significant difference according to Duncan's multiple range test at 5 % level.

Concerning plant water relations, TN leaves showed better Ψ_{PD} and Ψ_{MD} than the TR and TC ones (Table 2). Assuming that Ψ_{PD} reflects the soil moisture conditions along the rooting zone (Van Zyl 1987) and that all vines were grown in similar soil water availability, the higher values of TN vines may mainly reflect the comparative effectiveness of their root systems to acquire and transport water. In contrast, TC vines revealed low capacity in this aspect, which, associated to the possible high hydraulic resistance of the xylem vessel (Lovisolo and Schubert 1998), might be responsible for the lowest values of Ψ_{PD}. The g_s is an integrative parameter to reflect water stress experienced by plants, namely in grapevines (Flexas *et al.* 2002, Gómes-del-Campo *et al.* 2004, Souza *et al.* 2005). According to Düring and Loveys (1996), this high sensitivity of stomata can be explained by the heterobaric anatomy of grape leaves, which provokes, throughout the day, the development of water deficits in given portions of the leaf, and the consequent accumulation of abscisic acid in the guard cells, especially if the water potential in the leaf xylem and petiole is not very low.

Bulk parameters of leaf water relations (Table 2) revealed no significant differences in Ψ_π determined at full turgor, which suggests the same behaviour in degree of osmotic adjustment by net solute accumulation in response to water deficits promoted by the temporal imbalance between the absorption of water in the roots and xylem transport and rate of water loss by transpiration (Medrano and Flexas 2003). In contrast, certainly induced by changes in the cell wall properties, TC leaves exhibited lowest leaf cell wall elasticity (highest ε_{max}). This behaviour could be interpreted as a physiological mechanism which enables the leaves to change rapidly their water potential in response to small leaf water losses (Patakas *et al.* 1997).

The lower Ψ_{PD} and Ψ_{MD} in TC than in TN can help maintain water uptake from drying soil without undergoing a large tissue water deficit. In the TN leaves the opposite behaviour (lower ε_{max} and higher Ψ_{PD} and Ψ_{MD}) may also explain their larger aptitude to dry environments. In spite of the contrasting plant water relations, there were slight differences in g_s and P_N in TN and TC leaves. Rodrigues *et al.* (1993) refer that the response of stomata and carbon assimilation to drought was not directly associated with osmotic adjustment and occurred independently of bulk leaf tissue turgor. According to these authors, factors other than leaf water relations, related with the chemical signals coming from roots and/or the photosynthetic apparatus functionality, play probably a key role in carbon assimilation.

In conclusion, the typical lighter green leaves of TC are not a negative factor to grapevine physiology, since the photosynthetic productivity was only slightly affected, but an efficient adaptation strategy to environments characterised by high irradiance with a combination of water stress and high air temperature. From a viticultural point of view, the knowledge of these mechanisms of adaptation will support the selection of cultivars better adjusted to those environments. Future work should investigate the mechanisms of energy dissipation and xanthophyll composition in function of different typical colour leaves of the three studied cultivars and other equally important cultivars for vineyards regions with typical Mediterranean climate.

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