Water deficit effects on grapevine woody tissue pigmentations

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Abstract

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Water availability is an important environmental factor in viticulture. In a climate change context, vineyard management should be adapted to the new conditions. Drought-resistant rootstocks need to be selected. In this paper, reflectance spectroscopy is proposed as a new method to characterize the water stress effects on woody section pigmentations. Cabernet Sauvignon grafted on 4 different rootstocks (140Ru, 420A, M2 and M3) represented the plant material. Greenhouse controlled conditions allowed the comparison of well-watered (WW) and water-stressed (WS) plants. The physiological responses were characterized concerning daily water consumption, stem water potential, gas exchange, and plant growth. The water use efficiency was calculated and discussed as well. Spectroscopy analyses of woody sections indicated a major absorption band probably related to phenolic derivatives. Water stress produced characteristic spectrum modifications both in the Cabernet Sauvignon stem and in the rootstock xylem. These preliminary results encourage further studies addressed at the evaluation of drought-resistant genotypes, to distinguish their stress responses and to characterize the compositional aspects linked to drought tolerance.

Keywords: Cabernet Sauvignon; *Vitis*; reflectance spectroscopy; rootstock; water stress

Water stress events are becoming more and more frequent in the most important world's grape-growing regions (Chaves et al. 2007; Flexas et al. 2010; Medrano et al. 2014). This situation requires a special attention, because, beside the direct implications on winemaking industry managements, an increase in water use for viticulture could lead to a potential impact on freshwater conservation with obvious consequences on the environment (Hannah et al. 2013).

There are different ways to cope with drought stress limiting the environmental impact. First of all, it is important to target the agronomic practices to save water. Furrow irrigation should be replaced by drip irrigation (Tagar et al. 2012). Different canopy managements should be taken into consideration with the purpose of improving the water use efficiency (Medrano et al. 2012). Another possibility is to act on soil management aiming at reducing the soil evapotranspiration, for example, by using cultivated bare ground instead of cover crop. This would reduce water competition between cover crops and vines (Centinari et al. 2014). However, this choice should be carefully

considered because it could also favour erosion and soil pauperization (Reicosky, Forcella 1998).

Another way to deal with drought is to select new rootstocks resistant to water stress. In this context, rootstocks are generally expected to enable the scion to grow and to function normally when water supply is limited (Serra et al. 2013). To achieve this goal, rootstock selection aims at: improving the capacity to develop a large and deep root system with an increased aptitude to save water with a tight control of the scion transpiration (Chaves et al. 2010; Sommer et al. 2010); and enhancing the water uptake ability in dearth water conditions.

Woody tissues are known to have a characteristic brown colour. This colour is expected to change in relation to different factors such as chemical composition (Higuchi et al. 1994), species and age of tree (biological life) and wood (number of annual rings) (Klumpers et al. 1993). It is also affected by soil water availability (Klumpers et al. 1993), encouraging the development of colour-related methods in the drought stress studies. Nowadays, a number of methods are available to objectively describe the colour of specific surfaces. In particular, reflectance spectroscopy measures the radiation reflected by a surface at each wavelength of interest. In the visible region, simple reflectance spectra elaborations could give information concerning pigment contents. Recently, our group adopted this technique to develop indexes related to grape berry pigments including anthocyanins (Rustioni et al. 2013), melanin-like oxidized phenolics (Rustioni et al. 2014), chlorophylls and carotenoids (Rocchi et al. 2015).

The objective of this work is to characterize woody tissue pigmentation changes related to water stress. It aims at creating the basis for further studies devoted to characterize and quantify drought effects on grapevines. Future applications could be addressed to support drought resistant rootstock and cultivar selections.

MATERIAL AND METHODS

Experimental design. The experiment was performed in May 2014, under greenhouse environmental controlled conditions. Two-years old grafted vines were grown in 4 l pots, filled with 70% sand and 30% peat substrate. The selected scion was Cabernet Sauvignon. It was grafted onto 4 different rootstocks: two well-known and diffuse genotypes
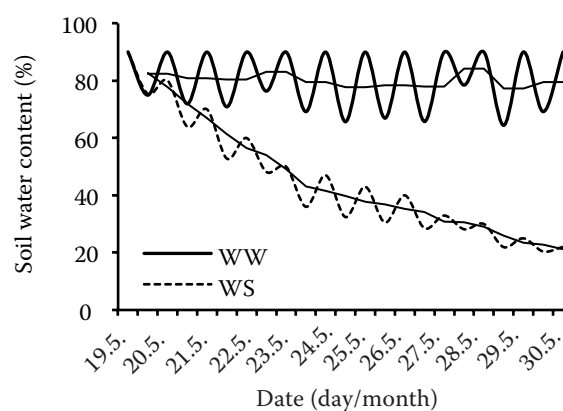

Fig. 1. Soil water content of well-watered (WW) and water-stressed (WS) pots during the experiment
every day the water soil capacity was totally (WW) or partially (WS) restored as reported by the wavy lines

(140Ru, 420A) and two new selections from the University of Milan (M2 and M3) and recently included in the Italian national register of grapevine varieties section rootstocks. Five replicates per rootstock-scion combination were monitored during the experiment: 2 plants were kept in well-watered condition (WW) and the other 3 replicates were subjected to an increasing water stress (WS) (Fig. 1).

All pots were arranged in a completely randomized design, allowing the comparison of the four different scion-rootstock combinations and their performance under drought stress (Table 1).

Rough inflorescences were eliminated as soon as they appeared.

The level of substrate water content (SWC) was controlled by irrigation. In this way, it was possible to quantify the daily water consumption (ml/pot). In details, every day, all pots were weighed to assess the evapotranspiration. Water content was recovered by irrigation to keep the plants at SWC levels around 80% for WW plants. WS vines underwent a controlled progressive drought stress until SWC of 22%.

Water stress characterization. Data were acquired both at the beginning and at the end of the experiment, considering all the 4 rootstock-scion combinations in all the stressed and well-watered replications.

In particular, the plant growth during the experiment was measured as the SGR – increase in stem length (cm), and root and leaves biomass accumulation (g of fresh weight). Furthermore, the leaf gas exchanges were measured by using a Ciras portable photosynthesis system (CIRAS-2; PP Systems,

Table 1. Statistical comparison between WW and WS plant parameters at the end of the water stress cycle (sampling time point 80% of soil water content for WW plants and 20% of SWC for WS plants)

	Variable (unit)	Well watered		Water stress		Sig.
		mean	std	mean	std	
Daily water consumption	DWC (ml)	272.5	39.6	59.4	29.4	0.000
Water potential	stem water potential (bar)	5.38	2.15	7.54	0.58	0.004
Gas exchanges	Ci (ppm)	199.8	15.5	324.4	117.8	0.009
	E (mmol·m^{-2}·s^{-1})	4.08	1.11	0.51	0.32	0.000
	Gs (mmol·m^{-2}·s^{-1})	191.6	60.4	17.3	11.4	0.000
	Pn (µmol·m^{-2}·s^{-1})	8.43	2.06	0.47	1.07	0.000
	Vdp (mbar)	23.8	2.4	29.9	3.8	0.001
	WUE intr. – Pn/Gs (µmol·m^{-2}·s^{-1}/mol·m^{-2}·s^{-1})	45.5	8.5	22.1	56.3	0.264
	WUE inst. – Pn/E (mmol·m^{-2}·s^{-1}/mol·m^{-2}·s^{-1})	2.11	0.42	0.99	2.53	0.234
Roots	root biomass (g)	20.7	6.4	15.7	3.3	0.033
	WUE roots biomass/DWC (g/l)	8.03	2.56	9.31	1.09	0.137
Leaves	leaf biomass (g)	18.6	3.5	15.2	2.9	0.030
	WUE leaf biomass/DWC (g/l)	7.05	0.64	9.01	0.98	0.000
Stem	stem growth (mm/day)	50.9	17.2	26.8	13.8	0.004
	SGR (cm/day)	3.27	2.14	0.51	0.60	0.000
	WUE stem growth/DWC (cm/l)	17.2	8.9	15.9	7.1	0.730

WW – well-watered; WS – water-stressed; SWC – soil water content; WUE – water use efficiency; intr. – intrinsic, inst. – instantaneous; SGR – shoot growth rate; Sig. – significance; std – standard deviation; DWC – daily water consumption; Ci – internal CO_2 concentration; E – evapotranspiration rate, Gs – stomatal conductance; Pn – photosynthetic activity; Vdp – air vapour pressure deficit

Amesbury, USA). This instrument allows monitoring of the different plants responses to drought in terms of: internal CO_2 concentration (*Ci*), photosynthetic activity (*Pn*), stomatal conductance (*Gs*), evapotranspiration rate (*E*) and air vapour pressure deficit (VPD). All measurements were taken around midday during the experiment. Table 1 reports values recorded at the end of the experiment.

These data were also elaborated as ratios (Table 1) to obtain indexes of the water use efficiency (WUE). These data refer to the ratio between growth and mean daily water consumption during the experimental period (10 days).

Finally, the stem water potential was quantified by a Scholander pressure chamber (Soilmoisture Equipment Corp., Santa Barbara, USA) in leaves kept bagged 1 h before measurements.

Reflectance analysis. For each plant (5 vines per graft combination), 3 trunk (rootstock) and 3 stem (Cabernet Sauvignon) sections were taken. Woody samples were fixed in an ethanol-water-formaldehyde-acetic acid (60:35:4:1) solution and stored until analysis. Before spectra recording, the samples were washed with pure water and dried at room temperature. In each trunk section, 4 xylem reflectance spectra were recorded in different position. Stem samples were much smaller, and, thus, only 2 measurements (one in each side) were recorded in each section.

Overall 360 reflectance spectra were obtained by a Jaz System spectrometer, completed by a Channel with a DPU Module and ILX511b Detector, OFLV-3 Filter, L2 Lens and 50 µm Slit as installed options (all from Ocean Optics, B.V., Dunedin, USA). A reflection probe QR600-7-VIS125 was coupled to the spectrophotometer. The instrument was set up with a NIR/Vis light source 4,095 power setting (both from Ocean Optics, B.V., Dunedin, USA) and the integration time was automatically corrected by the instrument. Each recorded spectra was calculated as the average of 20 measurements. Collected spectra ranged between 340 nm and 1,025 nm with a stepwise shift of about 0.3 nm. However, only the visible region of the spectra will be discussed in this paper. To exclude the lamp emission intensity variation at different wavelengths, the reflectance

spectra (*R*) were calculated as a ratio between the measured sample spectra and the reference blank spectrum obtained by a PTFE diffuse reflectance standard. A probe holder (Ocean Optics, B.V., Dunedin, USA) was included to ensure the analytical reproducibility: the distance between the sample surface and the probe was fixed at 12 mm.

Data elaboration. The reflectance spectra were converted to approximate to the quasi-linear relationship between pigment content and optical reflectance-based indices using reciprocal reflectance ($1/R$) spectra. The difference between WS and WW plant spectra was than calculated. The statistical significance of the differences were estimated for each wavelength according to the classical computation of the mean confidence intervals per $P = 0.95$, with: lower limit = $M - (t_{0.95})(s_m)$ and the upper limit = $M + (t_{0.95})(s_m)$

where:

M – average value; t – Student's t-test per $P = 0.95$; s_m – standard error (standard deviation/number of berries$^{1/2}$)

This analysis, as well as the ANOVA models, was performed by the SPSS® statistical software (Ver. PASW Statistics 19; SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

Water stress characterization

In general, the WS produced significant effects on the vines (Table 1); however, the plants were generally able to actively adapt to the changed conditions, keeping the water use efficiency unchanged or increased.

More in detail, WS plants showed a significantly lower amount of daily water consumption. This aspect is due to the lower stomatal conductance (*Gs*) and evapotranspiration rate (*E*).

Concerning the results of the stem water potential, the drought stress is confirmed by a significant decrease of the measured pressure.

Other important indicators of the significant drought stress obtained on the WS plants are summarized in Table 1 in the gas exchange subgroup. These data confirmed the stress, by some important parameters widely adopted as indicators of water deficiency in plants (Flexas et al. 2009; Hochberg et al. 2013; Meggio et al. 2014). From *Pn*, *Gs* and *E*, it is possible to quantify the intrinsic

and instantaneous WUE. In this case, no significant differences resulted from the treatments, underlining the general ability of the plants to maintain the physiological efficiency in the experimental stress conditions. These results are in agreement with the data published by Pou et al. (2012).

Considering the growth of the plants in terms of produced leaf and root biomasses and stem development during the experiment, the difference between the treatments is confirmed. Especially the stem growth took advantage from the well-watered conditions.

Concerning the WUE, results similar to that observed for the gas exchange were found for the growth parameters (expressed as root and leaf biomass and stem length produced per litre of water consumed). The WS vines demonstrated an ability to maintain the WUE. Concerning the root and leaf biomass produced, the efficiency in WS conditions resulted higher than the WW controls. Furthermore, considering the leaves, the WS significantly improved the efficiency in water use.

Drought effect on tissue pigmentation

Fig. 2 shows the average spectra of well-watered rootstock trunk xylem and Cabernet Sauvignon stems. It clearly appears a main absorption band in the blue region (around 500 nm). This band could be attributed to oxidized brown phenolic derivatives. As an example, Klumpers et al. (1993) found a considerably lower phenolic content in oak sapwood than in the darker heartwood. The detected main absorption band looks quite similar to the one already observed by Rustioni et al. (2014) in sunburned grape berries. In that case, it was attributed at melanin-like pigments (known to be mainly constituted by oxidized phenolics). It is also possible to hypothesize a participation of lignin to this absorption band, due to the characteristic brown colour of this class of molecules (Higuchi et al. 1994). However, to define the paper quality, the ISO (International Organization for Standardization) brightness index is determined at 457 nm and, in their article, Schmidt and Heitner (1993) underlined the importance of the spectrum range 300–500 nm for lignin absorption. For these reasons, we suppose the shoulder around 450 nm could be related to lignin chromophores. However, the low instrument of light intensity in the violet
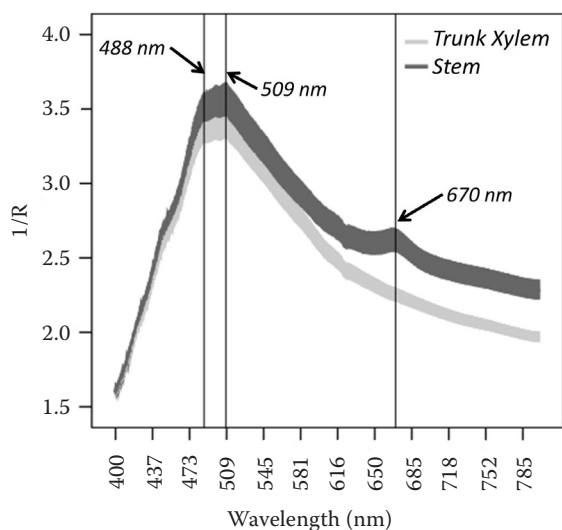
Fig. 2. Average reciprocal reflectance (1/*R*) spectra of rootstock trunk xylem and Cabernet Sauvignon stem

line thickness represents the mean confidence intervals per *P* = 0.95

region does not allow further considerations concerning lignin.

In Fig. 2, the main band seems to result by the sum of a number of small Gaussians. In fact, different shoulders could be identified in the asymmetrical main band. Moreover, also the absorption maximum ranges between 488 and 509 nm. This result is in agreement with the heterogeneity of the oxidative phenolic polymerization, in which free radical intermediates have a central role (WATERHOUSE, LAURIE 2006). Modifications in the polymeric structure would provide shifts in the specific absorption bands. Additionally, the absorption participation of lignin (a polymer as well) could also contribute to the band characteristics.

Considering the stems optical properties, it is also possible to underline a relatively small absorption band around 670 nm. Stem sections were collected close to the apex. Thus, this band is clearly related to chlorophyll traces, in agreement with the previous results (ROCCHI et al. 2015). Small shifts in the absorption maxima could be related to chlorophyll *a* and *b* mixture, as well as to differences due to the tissue characteristics (berries *versus* woody sections).

Fig. 3 reports the absorption variation (Δ1/*R*) related to the water stress in rootstock trunk xylem (a) and Cabernet Sauvignon stems (b). It is easy to note an opposite behaviour. In rootstocks, the stress produced an increase into the main band, while in

Cabernet Sauvignon stems the absorption intensity decreased. This behaviour was reproduced in all the grafting combinations. Thus, they indicate specific tissue answers to the drought stress. In particular, it is possible to hypothesize an accumulation of phenolic derivatives in the rootstock xylem, maybe also related to vessels occlusions and suberin deposition: all this behaviour is a typical plant adaptation to stressed conditions (POUZOULET et al. 2013). Considering Cabernet Sauvignon stems, the absorption decrease could be explained by the accumulation of sclereids which could screen the brown pigment reflectance detection (RAJAEI et al. 2013). Further studies (including chemical compo-
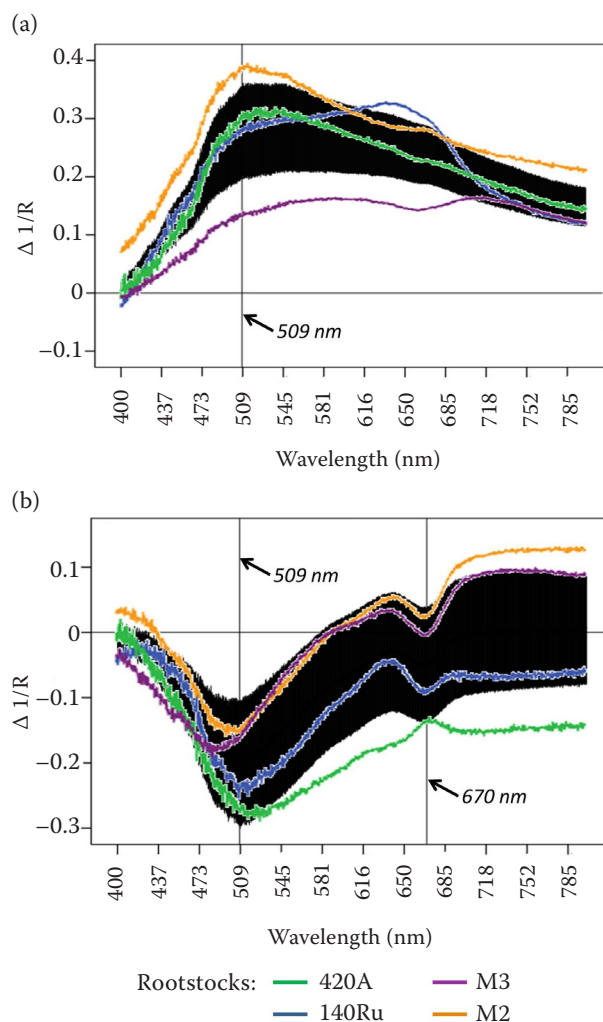

Fig. 3. Differences between water-stressed (WS) and well-watered (WW) plant spectra, (a) Cabernet Sauvignon stem data and (b) the rootstock trunk xylem values

line thicknesses represent the mean confidence intervals of the average strectum per *P* = 0.95

sition and microscopic analysis) will be necessary to confirm the spectral change origins.

CONCLUSION

Water stress significantly affects viticulture, especially in a global warmed condition. Rootstock selections could improve the water use efficiency in shortage situation. Phenotyping protocols, based on the drought physiological responses of the plant are essential. Reflectance spectroscopy could objectively describe some compositional changes related to water stress. Further studies will be necessary to clarify the differential behaviour of various genotypes. Experiments carried out in adult vines grown in open field could strongly improve the actual knowledges. Finally, these researches will produce phenotypic markers able to classify rootstock drought tolerances.

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