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Better Water Use Efficiency in Vineyard by Using Visible and Near Infrared Spectroscopy for Grapevine Water Status Monitoring

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The increasing water shortages are leading to develop new tools to better manage irrigation monitoring and scheduling for high water use efficiency. This results in a need of systems for rapid water status monitoring to better manage crop and irrigation scheduling for a high water use efficiency.
The objective of this preliminary work was to predict the grapevine water status in a rapid and non-destructive way using two portable optical devices (vis/NIR, 400-1000 nm; and NIR, 1000-2000 nm) for measurements directly on the leaves.
The measurements were performed on 72 leaf samples (cv. Cabernet Sauvignon) in a glasshouse under environmental controlled conditions (temperature and relative humidity). As references, a Scholander pressure chamber was used to measure the leaf water potential (Ψ) immediately after spectral acquisitions. Measurements were made around midday (10.00 to 14.00 hours, solar time) on three leaves for each plant chosen from the mid-upper part of the canopy. Moreover, the leaf total water content was quantified weighing each fresh leaf sample before the water potential measurement and after the drying process.
Principal component analysis (PCA) was performed on vis/NIR and NIR spectra to examine sample groupings, and partial least square (PLS) regression algorithm was used to correlate samples spectra and reference data. Regarding the vis/NIR results, PLS models showed for the prediction of leaf water potential R_{cv}^2= 0.67 and RPD = 1.7, and for the prediction of total water content R_{cal}^2 = 0.72 and RPD = 1.9. Slightly better results were obtained for NIR spectroscopy; PLS models achieved good prediction performance for total water content (R_{cv}^2 = 0.91 and RPD = 3.4) and acceptable results for leaf water potential (R_{cal}^2 = 0.63 and RPD = 1.8).
The study could provide the sector with portable optical systems for a quick evaluation of grapevine water status directly in field. Therefore, vis/NIR and NIR spectroscopy might support winegrowers for prompt decisions about the irrigation scheduling.

1. Introduction

Optimizing water consumption and improving its quality are considered central issues for the future also in the wine industry which places a significant role concerning water use. Grapevine growing regions are mostly characterised by water stress conditions due to high evaporative demand and low water availability. Nowadays irrigation scheduling in vineyards is performed through the measurement of soil moisture status using time consuming systems that are not easy to apply in field, may be affected by cumulative errors, may not be representative because of soil heterogeneity, and these methods increase moreover the irrigation costs (Acevedo-Opazo et al., 2010). Moreover, the increasing water shortages are leading to develop new tools to better manage irrigation control and scheduling for high water use efficiency. Developments in monitoring systems to precisely assess plant water status in the field or in greenhouse conditions will facilitate crop management (Costa et al., 2007). Cohen et al. (2005) investigated the potential of thermal images for an in-field estimation of the water status of cotton concluding that the selection of sunlit and shaded leaves cannot be achieved by using thermal images alone. The use of multispectral images in the visible and near infrared

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spectral ranges, coupled with thermal images, could allow to distinguish between sunlit and shaded leaves (Leinonen and Jones, 2004). Moreover, although the process of detecting sunlit and shaded leaves in the multispectral images was performed automatically, the registration and scale adaptation of the thermal and multispectral images were performed manually and so not easily transferable to real scale measurements.
Therefore, the applicability of visible (vis) and near infrared (NIR) spectroscopy as rapid techniques for the grapevine water status monitoring directly in the field is a challenge for the winemaking sector. A work published by De Bei et al. (2011) proposed the application of vis/NIR spectroscopy (300-1100 nm) for the prediction of water potential on Cabernet Sauvignon and Shiraz leaves; Santos and Kaye (2009) tested NIR spectroscopy (spectral range 1100 to 2300 nm) for the estimation of leaf water potential performing transmission measurements on Syrah, Merlot and Cabernet Sauvignon grapevines.
The aim of this work was to predict in a rapid and non-destructive way the water status of grapevine leaves using two portable optical devices (vis/NIR and NIR spectrophotometers) considering as reference measurements the leaf water potential and leaf water content. The possible final application would be an optical system that supports growers in determining rapidly water status to optimize the irrigation scheduling and saving water resources.

2. Materials and Methods

2.1 Sampling

A total of 216 spectral measurements were performed on leaves (cv. Cabernet Sauvignon) in the vis/NIR (400-1000 nm) and NIR (1000-2000 nm) range for water status monitoring directly in field using two portable optical devices. The measurements were conducted during the vegetative growth in July 2015 in a glasshouse under environmental controlled conditions (25-30 °C, RH 50-80 %). The irrigation was scheduled aiming to produce different stress status in order to create water status variability among grapevines.
Spectral measurements were performed directly in the glasshouse on three leaves for 12 plants, at different water status conditions (Table 1), selected for the analysis. As references, a Scholander pressure chamber (Scholander et al., 1965) was used to measure the leaf water potential (Figure 1) around midday (10.00 to 14.00 hours, solar time), on fully expanded and undamaged leaves for each plant chosen from the mid-upper part of the canopy (Cole and Pagay, 2015; Meron, et al., 1987). The water potential was measured immediately after spectral acquisitions on the same leaf.
The leaf water content was quantified weighing each fresh leaf sample before the water potential measurement and after the drying process.

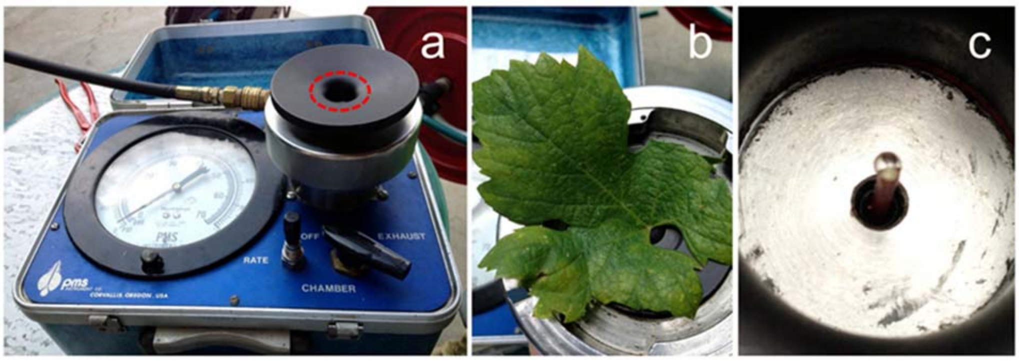

Figure 1. a) Details of Scholander pressure chamber; b) insertion of the leaf; c) the water leakage (end of analysis).

Tables 1 show ranges of leaf water potential (Ψ) and leaf water content values, respectively. In both cases three arbitrary levels ("1", "2", and "3") were created (Deloire and Heyns, 2011), identifying different ranges for each parameter. Regarding the low values of water potential (-1.66 – 2.10 MPa) corresponds to a high stress status (level 3), medium stress corresponds to level 2 (-1.21 - -1.65 MPa) and the lower stress status is represented by level 1 (-1.20 - -0.75 MPa). In addition, the parameter of the water potential is more related to the water status of the plant than the water content but is also used to determine water content and water status of plants (Hunt and Rock, 1989; Peñuelas and Filella, 1998). Concerning the water content levels, low values of water content (65.9-70.4 %) corresponds to a high stress status (level 1), while higher values of water content correspond to medium (level 2, 70.5-74.9 %) and low (level 3, 74.9-79.3 %) stress status. Water potential changes do not seem to be highly correlated to the water content. Some tests were performed about this issue on a part of the dataset available; no relevant correlation was found between water content and water potential (data not shown).

Table 1: Leaf water potential (LWP) and water content in sampled leaves.

Water status	LWP (MPa)	Level	Water content (%)	Level
Low	-1.20 - -0.75	1	65.9-70.4	3
Medium	-1.21 - -1.65	2	70.5-74.9	2
High	-1.66 - -2.10	3	74.9-79.3	1

2.2 Spectral acquisitions

Spectral acquisitions were performed on samples using a vis/NIR portable system operating in the wavelength range of 400-1000 nm (Jaz, OceanOptics, USA), and a NIR one working in the range 1000-2000 nm (TG-cooled NIR II, Hamamatsu Photonics, Japan).
Spectra were acquired in reflectance: light radiation was guided to the sample through a Y-shaped, bidirectional fiber optic probe (OceanOptics, USA) to the surface of the sample. The Y-shaped fiber allowed to guide light from halogen lamp (light source) to the sample while simultaneously collecting the radiation coming from the leaf and to guide it back to the spectrophotometer. The probe consists of a bundle of seven optical fibers (six illumination fibers around one read fiber, each one with a diameter of 600 µm). The tip of the optical probe was equipped with a soft plastic cap to ensure contact with sample skin during measurements, while minimizing environmental light interference. Moreover, a spectrally black surface was placed on the opposite side of the acquisition point to minimize differences in background reflectance.
Spectral resolutions were 0.3 nm and 8.0 nm for vis/NIR and NIR systems, respectively. Each spectral sample was obtained by averaging three acquisitions in three different points on the abaxial face of the same leaf. Each acquisition represents an average of five reflectance spectra.

2.3 Data analysis

Chemometric analyses of vis/NIR and NIR spectra were performed using the Unscrambler 9.8 software package (CAMO ASA, Oslo, Norway). Moving-average (15 nm point-wide window) smoothed spectra were used in a principal component analysis (PCA) to explore the possible clustering of sample spectra. Smoothing was applied to improve the signal-to-noise ratio to reduce the effects caused by the physiological variability of samples.
Moreover, a quantitative analysis was performed for the creation of a chemometric regression model for each reference parameter. The vis/NIR spectra were correlated with the leaf water potential and water content (reference parameters) using the partial least square (PLS) regression algorithm. In PLS regression, an orthogonal basis of latent variables is constructed one by one in such a way that they are oriented along directions of maximal covariance between spectral matrix X and response vector Y. This method ensures that the latent variables are ordered according to their relevance for predicting the Y variable. Interpretation of the relationship between the X data and the Y data (the regression model) is then simplified, as this relationship is concentrated on the smallest possible number of latent variables. The PLS method performs well when there is a large amount of correlation, or even collinearity, which is the case for spectral data of intact biological material (Nicolaï et al., 2007).
Cross-validation is an internal validation method, commonly used in the case of a small number of samples available for regression. With cross-validation, some samples are kept out of the calibration and used for validation procedure. This is repeated until all samples have been kept out once. In this case, a leave-more-out procedure of cross-validation was applied, using 10 elements for each cancellation group (Casale et al., 2008).
To evaluate model accuracy, the statistics used were the coefficient of determination in calibration (R_{cal}^2), coefficient of determination in cross-validation (R_{cv}^2), root mean square error of calibration (RMSEC), root mean square error of cross-validation (RMSECV), and ratio performance deviation (RPD, the ratio between the standard deviation of the response variable and the RMSE).
RPD ratio less than 1.5 indicates incorrect predictions, and the model cannot be used. RPD between 1.5 and 2 means that the model can discriminate low from high values of the response variable; a value between 2 and 2.5 indicates that coarse quantitative predictions are possible, and a value between 2.5 and 3 or above corresponds to good and excellent prediction accuracy, respectively (Sinnaeve et al., 2001).

3. Results and discussion

3.1 Spectral analysis

The average measured vis/NIR and NIR spectra are shown in Figure 2a and 2b, respectively. The average vis/NIR spectra showed a main absorption peak around 670 nm band, corresponding to the chlorophyll absorption peak (McGlone et al., 2002) and an absorption peak near 780 nm, representing the third overtone of OH bond stretching. Reflectance peaks centred around 540 nm and 760 nm are also noticeable, equal to the green reflectance peak and to the maximum reflectance peak, respectively. Regarding the NIR region, three main absorption bands are noticeable, at 1200 nm (combination band of the first overtone of the OH stretching and the OH bending band), in the band between 1400-1450 nm (first overtone of OH bond stretching), and at 1950 nm associated to the absorption of liquid H_2O (Carter and McCain, 1993).

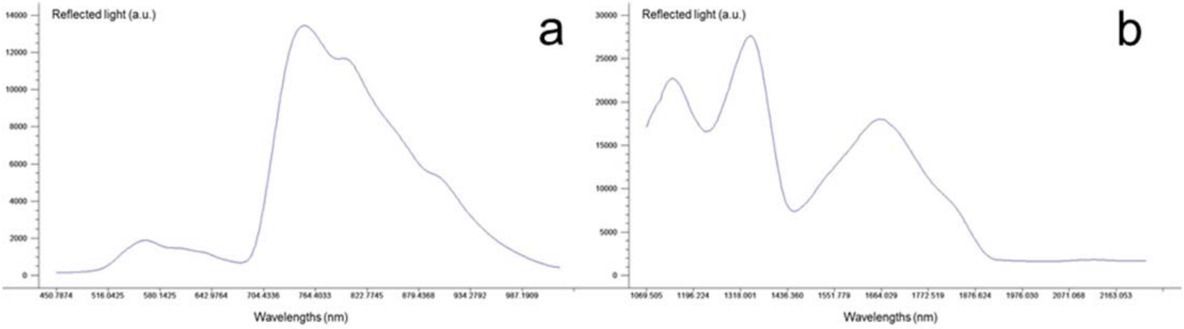

Figure 2: Average measured leaf spectra in the a) vis/NIR and b) NIR spectral range.

3.2 Results of qualitative analysis in the vis/NIR and NIR ranges

The explorative PCA conducted on 216 spectra of grapevine leaves resulted in two most significant PCs explaining 96 % of total data variance (PC1: 83 %; PC2: 14 %) for vis/NIR region. The PC1 vs. PC2 scores plot (Figure 3a) reveals that PC2 greatly accounts for separating different levels of leaf water potential. On the contrary, leaves with different levels of water potential are almost completely overlapping along the PC1 axis. Less evident is the separation among leaves with different water content in the PC1 vs. PC2 scores plot (Figure 3b), which shows that only a combination of these two PCs enables a poor separation of leaves with different water content levels. Hence, the PCA performed on vis/NIR spectra highlights a trend of stress level developments only considering the values of water potential (Figure 3a) and not for leaf water content parameter. In fact, the water potential is not closely related to the water content, and therefore not necessarily linked to the absorption of OH bonds. This behaviour probably occurs because in the vis/NIR range are noticeable only the absorption peak relating to the weak third overtone of the OH bond (760 nm). Stronger absorption bands of water are found around 1400-1440 nm and between 1900 to 1950 nm (Carter and McCain, 1993), i.e. in the NIR range. For this reason, the PC1 vs. PC2 scores plot deriving from PCA performed on NIR spectra (Figure 4) reveals fair samples discriminations on PC1 (73 %) based on water potential parameter (Figure 4a), showing stressed leaves at positive values of PC1 and non-stressed samples at negative values of PC1. In this spectral range a good samples separation is also noticeable as expected considering the water content (Figure 4b). In this case, the trend of water content change along PC2 (explaining 13 % of total data variance) highlighting stressed leaves at negative values of PC2 and non-stressed samples at positive values of PC2.

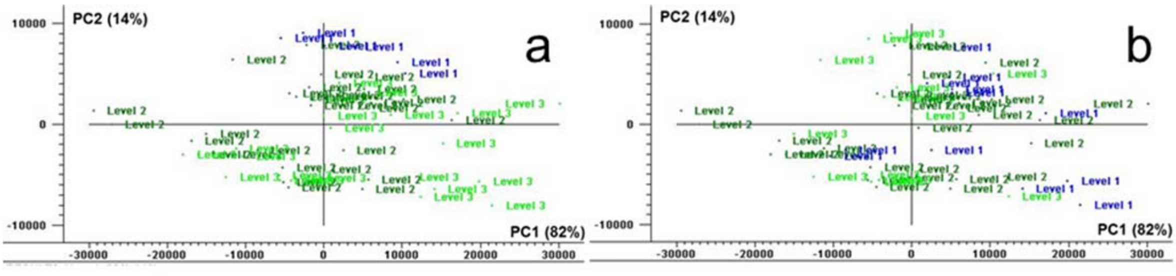

Figure 3. Score plot deriving from PCA of vis/NIR spectral samples, coloured by a) leaf water potential and b) water content

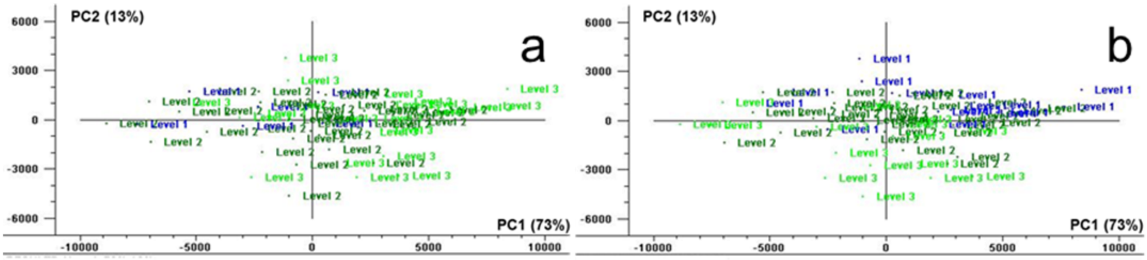

Figure 4. Score plot deriving from PCA of NIR spectral samples, coloured by a) leaf water potential and b) water content

3.3 Results of quantitative analysis

In Table 2 are summarized the statistics related to the PLS models obtained by vis/NIR spectroscopy for Ψ and water content prediction.
The vis/NIR models developed showed, for the prediction of leaf water potential R_{cv}^2 = 0.7 and RPD = 1.7, and of total water content R_{cv}^2 = 0.7 and RPD = 1.9. Slightly better results were obtained for NIR spectroscopy: PLS models achieved good prediction performance for total water content (R_{cv}^2 = 0.9 and RPD = 3.4) and acceptable results for leaf water potential (R_{cv}^2 = 0.6 and RPD = 1.8).
Overall results were satisfactory compared with findings reported in literature. De Bei et al. (2011) tested vis/NIR spectroscopy for the prediction of leaf and stem water potential of Cabernet Sauvignon and Shiraz leaves, obtaining regression models with correlation coefficients r ranging from 0.24 to 0.92 using a vis/NIR spectrometer (300-1100 nm). Santos and Kaye (2009) obtained models for estimate the leaf water potential, on leaves of Syrah, Merlot and Cabernet Sauvignon, with correlation coefficients r ranged from 0.87 to 0.95, in the NIR region from 1100 to 2300 nm.

Table 2: The statistics related to the PLS models obtained by vis/NIR (400-1000 nm) and NIR (1000-2000 nm).

						Calibration			Cross-validation		
Quality parameters	Spectral range (nm)	N°	Mean	SD	LV	R_{cal}^2	RMSECV	RPD	R_{cv}^2	RMSECV	RPD
Leaf water potential (MPa)	400-1000	65	15.08	3.35	8	0.79	1.53	2.19	0.67	1.94	1.73
Water content (%)		65	72.60	2.98	3	0.81	1.27	2.35	0.72	1.6	1.86
Leaf water potential (MPa)	1000-2000	65	15.27	2.89	8	0.81	1.24	2.33	0.61	1.83	1.58
Water content (%)		64	72.30	3.05	3	0.94	0.76	4.01	0.91	0.92	3.31

4. Conclusions

This preliminary work presented the applicability of NIRs spectroscopy as a rapid technique for the measure of leaf water status of grapevine leaves directly in field. Two instruments, vis/NIR and NIR spectrophotometers, were tested in green house conditions to estimate two parameters linked to leaf water status: leaf water potential and water content.
Through the elaboration of prediction models based on multivariate regression techniques, the prediction capabilities of the system for both parameters were evaluated and the obtained results were encouraging.
Further studies are desirable to improve these early results. For example, the number of samples of the available data set needs to be increased, and consequently the robustness of prediction models.
The goal for the future is to provide the sector with simplified systems for a quick evaluation of water status of grapevine directly in field avoiding the tedious and time consuming conventional techniques in the water status assessment of the vineyard, providing useful information for a better management of the irrigation scheduling.

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