Physiological indicators to assess water status in potted grapevine (Vitis vinifera L.)

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1. Introduction

Grapevine (Vitis vinifera L.) is a traditionally non-irrigated crop and generally classified as drought avoiding. The level of tolerance and the different physiological responses to drought are cultivar-dependent (Shelden et al., 2017). In the last ten years, with climatic change, irrigation was increasingly used in viticulture and experiments were performed for studying water stress and irrigation management. However, there is no general agreement on the most suitable vine water status indicators (Blanco-Cipollone, 2017; Behboudian and Singh, 2003; Cifre et al., 2005). Plant water status can be determined by using direct physiological indicators such as relative water content and water potential (Bennett, 1990; Sinclair and Ludlow, 1985) or indirect physiological indicators that describe processes induced by changes in plant water status, including variations in stomatal conductance, leaf temperature, and plant organ diameter as well as qualitative morphological alterations (Jones, 2004).

Many experiments have been performed to compare the early detection capability of the various physiological indicators in peach (Goldhamer et al., 1999; Remorini and Massai, 2003), plum (Intrigliolo and Castel, 2006), almond (Nortes et al., 2005), apple (Naor and Cohen, 2003), olive (Moria and Fereres, 2002) and lemon (Ortuno et al., 2006) but not in grapevine. To the best of our knowledge, the literature has reported so far only two comparisons among water potential indices (Choné et al., 2001; Williams and Araujo, 2002) as well as a comparison among the sensitivity of vegetative growth-based indicators, predawn leaf water potential and stomatal conductance (Pellegrino et al., 2005).

Pre-dawn leaf water potential (Carbonneau et al., 2004) is commonly used to determining water status in grapevine, while other indicators, such as stem water potential (Acevedo-Opazo et al., 2010; Choné et al., 2001; Liu et al., 1978; McCutchan and Shackel, 1992), midday leaf water potential (Girona et al., 2006), variations in plant organ diameter (Escalona et al., 2002; Intrigliolo and Castel, 2007), variations in stomatal conductance and net photosynthesis (Cuevas et al., 2006; Flexas et al., 2002; Maroco et al., 2002; Naor et al., 1997) and leaf temperature (Jones et al., 2002; Möller et al., 2006; Pou et al., 2000; Remorini and Massai, 2003).

Many physiological parameters were compared to identify the most sensitive and reliable indicator of grapevine water status. One-year-old potted grapevines (Vitis vinifera L., cvs. ‘Sangiovese’ and ‘Cabernet Sauvignon’) were studied under two irrigation treatments: 100% and 0% of daily water consumption. Measurements of pre-dawn (PD) and midday (MD) leaf water potential ($\Psi_w$), MD stem water potential ($\Psi_S$), leaf temperature ($T°L$) and stomatal conductance ($g_S$) were taken throughout twenty days and analyzed in conjunction with climatic data, relative cumulative sap flow (RCSF) and the maximum daily shrinkage (MDS) of the vine stock. Physiological indicators showed substantial differences in sensitivity. The first indication of changes in vine water status was the increase of MDS and the decrease of $g_S$. MDS and RCSF revealed significant differences between the two irrigation treatments even when $PD\Psi_w$ up to now widely accepted as the benchmark of water status indicators, did not show any significant variation. Measurements of water potential showed $\Psi_S$ to be a better indicator of vine water status than $\Psi_w$ and $T°L$. In conclusion, we classified the tested indicators according to a descending order of their early detection capability: $g_S = MDS > RCSF > PD\Psi_w = MDS > T°L > MD\Psi_w$. 

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ABSTRACT

Many physiological parameters were compared to identify the most sensitive and reliable indicator of grapevine water status. One-year-old potted grapevines (Vitis vinifera L., cvs. ‘Sangiovese’ and ‘Cabernet Sauvignon’) were studied under two irrigation treatments: 100% and 0% of daily water consumption. Measurements of pre-dawn (PD) and midday (MD) leaf water potential ($\Psi_w$), MD stem water potential ($\Psi_S$), leaf temperature ($T°L$) and stomatal conductance ($g_S$) were taken throughout twenty days and analyzed in conjunction with climatic data, relative cumulative sap flow (RCSF) and the maximum daily shrinkage (MDS) of the vine stock. Physiological indicators showed substantial differences in sensitivity. The first indication of changes in vine water status was the increase of MDS and the decrease of $g_S$. MDS and RCSF revealed significant differences between the two irrigation treatments even when $PD\Psi_w$ up to now widely accepted as the benchmark of water status indicators, did not show any significant variation. Measurements of water potential showed $\Psi_S$ to be a better indicator of vine water status than $\Psi_w$ and $T°L$. In conclusion, we classified the tested indicators according to a descending order of their early detection capability: $g_S = MDS > RCSF > PD\Psi_w = MDS > T°L > MD\Psi_w$. 

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Xylem sap flow measurement has been proposed to monitor the plant water consumption (Ginestar et al., 1998; Yunusa et al., 2000), and together with physiological and physical indicators adequately estimate crop water status (Cifre et al., 2005; Nadezhdina, 1999; Remorini and Massai, 2003).

The aim of the present study was to assess the most sensitive and reliable indicator of grapevine water status. The outcomes of this study will be useful from a scientific advancement perspective as well as for the several practical implications in irrigation management. Specifically, we compared xylem sap flow and trunk diameter fluctuations with a wide range of water stress indicators in two potted grapevine cultivars during water stress cycle.

2. Materials and methods

2.1. Plant material and study site

Trials were conducted at the experimental site of University of Pisa on vines (Vitis vinifera L.) cvs. Sangiovese (SG) R80 grafted onto S.O.4-2GM and Cabernet Sauvignon (CS) CL. 337 grafted onto 1103P-ISV1. In winter, one year-old vines were potted into 17L pots with soil:peat-perlite (2:1:2) medium and let grow outdoor. Vines were pruned leaving only a two-bud spur, and, after bud burst, a single shoot was left per vines. By the end of June, just before starting the experiment, vines were divided into two uniform groups, each of 40 pots. The first group, formed by controls (20 vines for each rootstock combinations), was watered daily, whereas the second group, formed by stressed plants, was not watered. Every day of the trial, at sunset, irrigation water was added in order to maintain the soil water content in the root zone around 90% of field capacity; water volume was determined by weighing each pot before and after irrigation to assess the daily water consumption. Starting from the beginning of the treatment (June 20th) the following water status indicators were daily measured: relative cumulated sap flow (RCSF), maximum daily shrinkage (MDS) of the vines stock, predawn (PD) and midday (MD) leaf water potential ($\Psi_w$), midday stem water potential ($\Psi_s$), stomatal conductance ($g_s$) and leaf temperature ($T_L$).

Air temperature, relative air humidity, solar radiation, rainfall, and reference evapotranspiration rate were measured by an automated meteorological station (WeatherHawk, Logan, Utah, USA), placed in proximity of the experimental site.

2.2. Water status indicators

2.2.1. Plant water potential

$\Psi_w$ was measured before dawn (PD) and in the hottest part of the day (MD), while $\Psi_s$ was determined only at MD, thus leading to three indicators (PD$\Psi_w$, MD$\Psi_w$, and MD$\Psi_s$).

$\Psi_w$ and $\Psi_s$ were measured using a Scholander type (Scholander et al., 1965) pressure chamber (Technogas, Pisa, Italy). Pressurization rate was 0.2 MPa every 30 s. $\Psi_w$ was measured on one leaf per vine. Leaves, well exposed to sunlight, were cut off halfway along the stalk and immediately processed (Turner and Long, 1980). $\Psi_s$ was measured on one leaf per vine, previously wrapped in aluminium foil and encased in polyethylene bags at least 1 h before measurement. In all cases, leaves were placed in the chamber within a few seconds from excision.

2.2.2. Leaf temperature

$T_L$ was determined at 10.00 h and 12.00 h, using a Cyclops Compac 3 infrared portable thermometer (Land Infrared Ltd., Sheffield, England). The sensor was placed at a distance (~10 cm) from the leaf blade to obtain a target area of 35 mm in diameter. The entire area, detected by the sensor, was occupied entirely by a single leaf in full sunlight. $T_L$ measurements were performed on 3 leaves per vines.

2.2.3. Maximum daily shrinkage

MDS indicator was calculated starting from trunk diameter fluctuations (TFD) measurements. Specifically, TDF was measured by a micrometric system, based on permanent measurement, with 10 µm accuracy. Twelve DF 2.5 linear transducer sensors (Solartron Metrology, Bagnor Regis, UK), mounted on trunk sensor carriers and connected to a CR10X Campbell datalogger (Campbell Scientific Inc., Logan, Utah, USA), were applied to the trunk of 12 selected vines (3 control and 3 stressed for each cultivar/rootstock combination). Readings were taken every 15 s and computed every 30 min to output mean values. TDF diurnal trends also allowed calculation of the MDS, by evaluating the difference between the maximum diameter, usually observed in the early morning, and the minimum diameter reached in mid-afternoon (Huguet et al., 1992).

2.2.4. Relative cumulated sap flow

SF measurements were carried out using a heat balance system applied in a portion of the branch (Sakuratani, 1981; Baker and Bavel, 1987; Steinberg et al., 1989). Gauges were installed on a regular portion of the main stem with no swellings or lumps that could weaken the contact between bark surface and the heater or thermocouples. Any loose bark was carefully removed by a blade (Smith and Allen, 1996). The system consisted of 12 SGA10 or SGA13 sensors (Dynamax Inc., Houston, Texas, USA) placed on trunks (3 control and 3 stressed vines for each cultivar/rootstock combinations). Sensors were installed during the hottest part of the day (when the diameter shrinks to its smallest size), and the bark was slightly thinned, having the care of not affecting the epidermis, to increase gauge sensitivity and ensure good contact between bark and gauge. In addition, G4 silicone type paste was applied to achieve good thermal contact and conductivity (Smith and Allen, 1996). Sensors and adjacent trunk portions were protected from solar radiation by aluminium foil to avoid the development of external thermal gradients.

The sensors were connected to a CR7 Campbell datalogger (Campbell Scientific Inc., Logan, Utah, USA). Readings were taken every 15 s and processed with Dynamax DGSF 5.0 software to supply accumulated values every 30 min (Steinberg et al., 1989). Vine water consumption was calculated from daily accumulated SF values.

2.2.5. Stomatal conductance

Stomatal conductance ($g_s$) at saturating light (namely at > 1200 µmol m$^{-2}$ s$^{-1}$ over the PAR waveband) were measured on medial leaves, using a portable infrared gas analyser Li-Cor 6400 (Li-Cor Inc., Lincoln, NE, USA) operating at 34 ± 0.5 Pa ambient CO$_2$. $g_s$ was determined at 11.00 h on 3 leaves per plant.

2.3. Statistical data analyses

One-way factorial analyses of variance (ANOVA, Statgraphics Centurion) was used to test the treatment effects on water status physiological indicators. Significant differences between treatments means were evaluated with LSD test. All values shown represent the mean ± standard error.

3. Results

Water balance of potted vines was monitored throughout the experimental period (Fig. 1). In the 3rd day of treatment, the pots with non-irrigated vines showed a statistically lower gross mass. The evapotranspiration of non-irrigated vines remained linear for the first 10–11 days, constantly decreasing in the following period.

Physiological indicators were measured throughout the experimental period. Table 1 shows the one-way ANOVA analysis during the treatment.

Experimental period patterns of PD$\Psi_w$, MD$\Psi_w$ and MD$\Psi_s$ are shown in Fig. 2. As can be seen, the PD$\Psi_w$ values of irrigated vines were
the highest and assumed a constant value around -0.02 MPa throughout the experiment period. In both cvs, significant differences were observed between the treatments after 8 days (Fig. 2 and Table 1), when PDΨw values for non-irrigated vines dropped to −0.05 MPa. Subsequently, non-irrigated SG values were constant for a week and then decreased down to −0.25 MPa, while CS values constantly decreased over the whole period. MDΨw values for both treatments were close to −1.0 and −1.1 MPa, respectively for CS and SG, and the differences between the treatments became significant only after 18 and 20 days (around 0.2 MPa), respectively for CS and SG (Fig. 2 and Table 1). Measurements of MDΨs did not show statistically significant differences between irrigated and non-irrigated vines until day 9 for SG and day 10 for CS. Afterwards, MDΨs average measurements showed statistically lower values for non-irrigated vines. MDΨs values for irrigated vines were close to −0.25 MPa throughout the experimental period, whereas gradually decreased for non-irrigated vines, reaching the minimum value (−1.0 MPa) at the end of the trial (Fig. 2 and Table 1).

In regards to the stomatal conductance, well-watered vines exhibited gs at a constant value of about 0.18 and 0.15 mol m⁻² s⁻¹ for SG and CS respectively throughout the experimental period, whereas in non-irrigated vines gs decreased to 0.02 mol m⁻² s⁻¹ after 5–6 days (Fig. 3 and Table 1). The non-irrigated cultivars showed a different

Table 1
One-way analysis of variance based on the Fisher’s LSD method. The significance level of the differences between treatments in different days is marked with * (95% interval), ** (99% interval) or ns (not significant). Predawn leaf water potential (PDΨw), midday leaf water potential (MDΨw), midday stem water potential (MDΨs), maximum daily shrinkage (MDS) and relative cumulative sap flow (RCSF) data represent the average of at least three measurements; stomatal conductance (gs) and leaf temperature at 10.00 h and 12.00 h (10hT°L and 12hT°L, respectively) data represent the average of at least nine measurements. All data were measured in Sangiovese cv. (SG) and Cabernet Sauvignon cv. (CS) potted vines.

| Indicator | Cvs. | Days of treatment | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|-----------|-----|------------------|---|---|---|---|---|---|---|---|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| PDΨw SG  | ns  | ns               | ns| ns| ns| ns| ns| ns| ns| * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| CS        | ns  | ns               | ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns |
| MDΨw SG  | ns  | ns               | ns| ns| ns| ns| ns| ns| ns| * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| CS        | ns  | ns               | ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns |
| MDΨs SG  | ns  | ns               | ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns |
| CS        | ns  | ns               | ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns |
| MDS SG   | ns  | ns               | ns| ns| ns| ns| ns| ns| ns| * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| CS        | ns  | ns               | ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns| ns |
| RCSF SG  | ns  | ns               | ns| ns| ns| ns| ns| ns| ns| * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| CS        | ns  | ns               | ns| ns| ns| ns| ns| ns| ns| * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| gs SG    | ns  | ns               | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| CS        | ns  | ns               | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| 10hT°L SG | ns  | ns               | ns| ns| ns| ns| ns| ns| ns| * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| CS        | ns  | ns               | ns| ns| ns| ns| ns| ns| ns| * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| 12hT°L SG | ns  | ns               | ns| ns| ns| ns| ns| ns| ns| * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| CS        | ns  | ns               | ns| ns| ns| ns| ns| ns| ns| * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
decreasing pattern: statistically significant differences starting the 5th day for SG and 6th for CS and $g_s$ for SG decreased at a lower rate with respect to CS.

Non-irrigated plants showed slower growth than controls. This trend was detected early, 5–6 days after the beginning of treatment (data not shown). The range of MDS was more pronounced in non-irrigated as compared to irrigated vines (Fig. 4) starting at the 5th-6th days for SG and CS, respectively, while at the end of experimental period differences between treatments became not significant (Table 1).

Vines with no symptoms of water stress showed an RCSF rate increase during the day from dawn to the hottest time (data not shown). RCSF, expressed as relative transpiration (Valancogne et al., 1997), showed differences between treatments starting from the 6th–8th day, for CS and SG respectively (Fig. 5 and Table 1).

$T_{L}$ measured at 10.00 h and 12.00 h (Fig. 6) shown differences between treatments starting from day 10, while differences were recorded for SG after 9 days of water stress (Table 1).

Fig. 7 provides a synoptic view of all indicators trends during the treatment. Specifically, the figures show the ratios computed between the mean indicator value for the well-watered vines and the mean indicator value for the droughted vines, plotted with respect to the day of treatment. A logarithmic scale was employed for the Y-axis in order to improve the readability at low ratio values. This figure, coupled with Table 1, enable assessment of the early detection capability of the various indicators.

Fig. 8 shown box-and-whiskers plot of all examined indices for both well-watered and stressed vines. Each box spans from the 25% percentile to the 75% percentile (and, thus, its extent is equal to the interquartile range) of the corresponding data. The median (50% percentile) of the data is plotted as a horizontal line within the box. In this plot, the two whiskers emanating from the box extend, in each direction, to the minimum and maximum data values. This kind of plot highlights data dispersion and skewness, thus providing interesting insights for comparing distributions of indicators between the two sets of CT and ST vines. The more the ST box is separated from the CT box, the more the given index is sensitive at detecting water stress, such as $g_s$ and RCSF indicators. Conversely both $10T_{L}$ and $12T_{L}$ exhibit CT and ST boxes that overlap for the majority of data values, thus indicating a lower sensitivity of the corresponding indicators. A good indicator should also provide a more compact distribution (and thus, a small shrunk box) for the CT case than for the ST case, such as $PD\Psi_W$ and $MD\Psi_S$. $MD\Psi_W$ of the CT indicator has a variability as great as – or greater than, for SG – the ST; however, especially for the CS case, the two medians are considerably separated.
4. Discussion

So far \( \Psi_w \) has been widely accepted as the benchmark of plant water status indicators. Unlike other plant water status indicators, \( \Psi_w \) measured at pre-dawn is a true soil-plant-atmosphere balance indicator that is independent of micrometeorological conditions (Katerji et al., 1988). However, \( \Psi_w \) has been shown to be not ideal in detecting early phases of plant water stress as well as poorly sensible as grapevine water status indicator (Cifre et al., 2005; Schultz, 2003).

Among the set of water status indicators we compared, we found (Table 1 and Fig. 7) that the earliest indication of water stress is provided by adjustments in stomatal conductance, in accordance to previously reported data (Flexas et al., 2002; Schultz, 2003), which succeeded even when \( \Psi_w \) failed. This early detection capability of \( g_s \) is likely to be due to the tight regulation of stomatal closure in grapevine in response to very mild soil water deficit (Cifre et al., 2005).

Furthermore, data show that the changes in the diameter of the trunk are a good water status indicator, in accordance to previously reported data in grapevine where vine growth was strictly related to the daily sap flow (Escalona et al., 2002). MDS values in water-stressed vines were higher than in irrigated ones and then became lower under severe water stress conditions, confirming Huguet et al. (1992) results. We found significant differences in daily shrinkage, confirming that increasing amounts of water reserves were recruited to sustain leaf transpiration with the progression of water stress (Escalona et al., 2002; Remorini and Massai, 2003).

T\(_L\) data confirm that this indicator is not useful to early detection of water status variations. Analysis of data shown that this indicator depends on the solar radiation (data not shown): differences were not recorded in cloudy days, such as day 13.

The distribution analysis performed with the box-and-whiskers plot (Fig. 8) also indicate a low sensitivity of T\(_L\) indicators with respect to water status as opposed to the much greater one exhibited by \( g_s \) and the other indicators.

5. Conclusions

The compared physiological indicators showed different early detection capability in estimating the water status of grapevine. \( g_s \) and MDS resulted as good indicators in terms of both early detection and sensitivity, whereas T\(_L\) and m\( \Psi_w \) were found to have low sensitivity. Finally, our study allowed to classify the tested physiological indicators of grapevine’s water status in the following decreasing scale of sensitivity: \( g_s = MDS > RCSF > PD\Psi_w = \Psi_S > T_{L} > MD\Psi_w. \)