Hydric behaviour and gas exchange in different grapevine varieties (*Vitis vinifera* L.) from the Maule Valley (Chile)

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In the near future, stomatal behaviour will be crucial to counteract conditions arising from climate change. Grapevine varieties are classified as either isohydric or anisohydric, depending on the sensitivity of stomata to water deficit and on their water potential homeostasis. However, the great variability observed in different studies indicates that a continuum exists in the range of stomatal sensitivity to water stress. Thus, more knowledge about the hydric behaviour and the gas exchange of isohydric and anisohydric grapevine varieties under different water conditions could lead to the development of irrigation strategies oriented at improving water-use efficiency, yield and berry composition. In this study, research was conducted in order to characterise the stomatal regulation of four different *Vitis vinifera* L. varieties, namely Pinot noir, Sauvignon blanc, Chardonnay and Merlot, according to soil water status. Measurements of leaf gas exchange, together with measurements of stem water potential (Ψs) and leaf water potential (Ψl), were taken during two seasons. Under conditions of water stress, Chardonnay and Merlot reached a minimum Ψs of -1.67 and -1.68 MPa respectively, and higher levels of water-use efficiency ($A_{N/}g_s$), of 62.3 and 69.7 µmol $CO_{2/}$mol H_2O respectively. In Sauvignon blanc and Pinot noir, the minimum Ψs was -1.26 and -1.40 MPa respectively, with lower levels of $A_{N/}g_s$ (53.1 and 50.5 µmol $CO_{2/}$mol H_2O, respectively). Under conditions of water stress (Ψl < -0.9 MPa and Ψs < -0.6 MPa), all varieties had a significantly increased $A_{N/}g_s$[1], despite a significant reduction in gas exchange. Therefore, the hydric behaviour and gas exchange observed in this study suggest that Chardonnay and Merlot could be characterised as anisohydric varieties, as they present less sensitive stomatal control, while Pinot noir can be classified as a near-anisohydric variety and Sauvignon blanc as an isohydric variety. New investigations should consider other characteristics of the varieties to classify them better.

INTRODUCTION

Recently, viticultural management has experienced a series of modifications due to the increase in temperatures, which has had direct effects on grapevine ripening and, consequently, on berry and wine quality (Van Leeuwen & Darriet, 2016). The effects of climate change on winemaking can vary according to the style of wine produced and by geographical location, with milder effects expected for coastal regions (Jones *et al*., 2005; Duchêne *et al*., 2010; Verdugo-Vásquez *et al*., 2019). Model outputs have predicted an average warming of 2ºC in the next 50 years for global wine-producing regions (Jones *et al*., 2005). Advances from eight to 11 days for budburst and from 16 to 24 days for véraison have been predicted for the end of the 21st century for white grapevine varieties cultivated in Alsace (Duchêne *et al*., 2010). A decrease in the length of each phenological stage in grapevines may yield a precocity towards the end of the 21st century of 40 days earlier than the current timeframe (Ollat & Touzard, 2014; Sgubin *et al*., 2018). Thus, grapevines have undergone modifications in their physiological behaviour that have affected vegetative growth (Gómez del Campo *et al*., 2002; Lebon *et al*., 2006), berry development (McCarthy, 2000; Ojeda *et al*., 2001), and the maturity and organoleptic composition of the grape berry at harvest (Koundouras *et al*., 1999; Ojeda *et al*., 2002). Thus, the observed climatic effects would be modifying both the spectrum and the distribution of the currently used grapevine varieties (Popescu *et al*.,

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2009), and would imply the implementation of adequate measures to reduce the negative effects through adjustments and changes in agricultural systems.

Soil water deficit and vapour-pressure deficit (VPD) are the most important environmental factors affecting stomatal closure in the Mediterranean area (Klein, 2014). Pou *et al*. (2008) reported that stomatal conductance (g_s) and the rate of transpiration (E) were strongly affected by VPD in a manner that is dependent on irrigation treatment. Depending on the stomatal control strategy followed in response to water deficit, grapevine varieties can behave as isohydric or anisohydric plants (West *et al*., 2012). In isohydric varieties, g_s responds to a greater extent to the changes in VPD. This high stomatal sensitivity of isohydric plants is usually associated with higher levels of abscisic acid in the xylem sap, and with hydraulic responses (Tardieu & Simonneau, 1998; Soar *et al*., 2006). In contrast, anisohydric grapevines have less control over Ψl, and the magnitude of Ψl decreases as VPD increases, reaching much lower values of Ψl in stressed vines than in grapevines growing under favourable water conditions. In this case, stomatal closure in these grapevines is associated with root signals or with the anatomical architecture related to stress conditions (Tardieu & Simonneau, 1998; Collins *et al*., 2010; Rogiers *et al*., 2011; Gerzon *et al*., 2015). Anisohydric grapevines present a substantial depression of their Ψ during drought, also showing some degree of tolerance to water stress (Bucci *et al*., 2005; Pou *et al*., 2012). Pou *et al*. (2012) reported that anisohydric behaviour results in better performance under moderate water stress and recovery than isohydric-behaved grapevines (Bucci *et al*., 2005; Pou *et al*., 2012).

A better knowledge of the hydric behaviour and gas exchange of isohydric and anisohydric grapevine varieties managed under different water conditions could lead to the development of irrigation strategies oriented to improving water productivity, yield and berry composition under the current effects of climate change. Therefore, the aim of this work was to characterise the vine water potential and gas exchange in four grapevines varieties (isohydric and anisohydric) managed under different water conditions in the Maule Valley, Chile over two consecutive seasons.

MATERIALS AND METHODS

Experimental site and plant material

The field trial was conducted in an experimental vineyard (cv. Chardonnay, Merlot, Pinot noir and Sauvignon blanc) belonging to the Vine and Wine Technological Centre of Talca University during the 2011/2012 and 2012/2013 seasons. The vineyard is located in Panguilemo, Talca, Maule Valley, Chile (35°22.2' south, 71°35.39' west, and 121 metres above sea level). The ungrafted grapevines were planted in 2006, trained to a vertical shoot position trellis system and were pruned into two bilateral spur cordons. Planting density was about 5 000 vines ha^{-1}, with grapevine spacing between rows and within the row of 2.00 m × 1.00 m and an east-west orientation. The soil texture was clay loam with a rooting depth of 150 cm, and the soil is classified as part of the Talca series (Thermic Ultic Haploxeralf). Bulk density, field capacity, wilting point and available water along the soil were 1.36 g cm^{-3}, 0.31 m^3 m^{-3}, 0.13 m^3 m^{-3} and 0.18 m^3 m^{-3} respectively. More information concerning soil and crop management carried out in the experimental field are available in Pañitrur-De la Fuente *et al*. (2018).

Information about weather conditions was recorded by an automatic weather station installed 50 m from the trial plot. Maximum and minimum temperature in the 2011/2012 grapevine growth season (from September to April) was 34.6ºC and 0.0ºC respectively, while in the 2012/2013 season it was 34.5ºC and 0.9ºC respectively. Average temperature in the season was 16.9ºC and 16.7ºC for the 2011/2012 and 2012/2013 seasons respectively. The rainfall registered for the phenological cycle in the 2011/2012 season reached 31.8 mm, and was concentrated in the spring months. The rainfall registered for the 2012/2013 season reached 152.4 mm, was concentrated in November, and there was occasional rain throughout the summer, unlike the 2011/2012 season, when there was no precipitations during the summer. The reference evapotranspiration (ET_o) was estimated using the Penman-Monteith equation (Ahumada-Orellana *et al*., 2018). ET_o calculated for the 2011/2012 season was 1 037 mm, and for the 2012/2013 season it was 939 mm. In the 2011/2012 season, 1 438 chilling hours were accumulated, while in the 2012/2013 season 1 243 chilling hours were accumulated. During the 2011/2012 season, the accumulation of degree days was 1 375ºC, while the accumulation of degree days during the 2012/2013 season was 1 311ºC. Mean VPD during the experiment in the 2011/2012 season was 1.05 kPa, while in the 2012/2013 season it was 0.95 kPa. Additional information is presented in Figs 1 to 4 of the supplementary material.

Experimental design

The experimental design was a randomised complete block divided into two plots (treatments), in which the four *Vitis vinifera* L. varieties under study were arranged: Chardonnay, Merlot, Pinot noir and Sauvignon blanc. During the first season, one of the two blocks was managed under optimal water conditions (without irrigation restriction), while the other was managed with progressive water restrictions until reaching severe water deficit in the vines (leaf water potential < -1.4 MPa) (Van Leeuwen et al. (2009). During the second season, differential irrigation management was not carried out as in the first season; instead, all the plants were irrigated with the same water load. Despite this, plants presented differences in their water status in the second experimental year, thus presenting a wide range of water stress within the experiment. A total of eight treatments (variety × water condition) were arranged in the vineyard, with five plants per treatment. The selected grapevines presented good phytosanitary conditions and were homogeneous in vegetative growth and productivity.

Gas exchange measurement

An infrared gas analyser, model LI 6400 (Li-cor, Lincoln, Nevada, USA), was used to measure stomatal conductance (g_s), transpiration (E) and net CO_2 assimilation (A_N). In addition, the intrinsic water-use efficiency (A_N g_s^{-1}) was calculated as the ratio between A_N and g_s according to Medrano *et al*. (2014). The gas exchange measurements were carried out in the middle zone (6th leaf from the tip on fruit-

bearing shoots) on the northern side of the canopy between 12:00 and 14:00 in five different plants per treatment on fully sunny days. The original position of the selected leaves in the canopy was not changed and the same light regime was maintained, thus ensuring that the leaves were exposed to full sunlight (PAR > 800 ***µmol*** m^{-2} sec^{-1}). Measurements were taken every 15 days between December (one month before véraison) and April (near harvest) in each growing season.

Plant water status

Stem water potential (Ψs) and leaf water potential (Ψl) were measured using a pressure chamber (PMS Instrument Co., model 600, Corvallis, Oregon, USA) according to the protocol stated by Acevedo-Opazo *et al*. (2013) and Jara-Rojas *et al*. (2015). Briefly, five fully expanded and sun-exposed leaves per treatment were wrapped in plastic transparent film and aluminium foil for at least 2 h, thus achieving an equilibrium between leaf and plant xylem. Ψl measurement were performed on uncovered leaves at the same time that Ψs and gas exchange measurements were taken (between 12:00 and 14:00). To define the level of water stress in this study, data were divided into five ranges according to the thresholds proposed by van Leeuwen *et al*. (2009): i) no water deficit (Ψl > -0.9 MPa; Ψs > -0.6 MPa); ii) mild water deficit (-0.9 ≤ Ψl ≤ -1.1 MPa; -0.6 ≤ Ψs ≤ -0.9 MPa; iii) moderate to mild water deficit (-1.1 ≤ Ψl ≤ -1.3 MPa; -0.9 ≤ Ψs ≤ -1.1 MPa; iv) moderate to severe water deficit (-1.3 ≤ Ψl ≤ -1.4 MPa; -1.1 ≤ Ψs ≤ -1.4 MPa; v) severe water deficit (Ψl and Ψs < -1.4 MPa).

Statistical analysis

The statistical analysis in relation to the parameters analysed was performed by ANOVA, using Centurion XVI.I (Statgraphics Technologies; The Plains, Virginia, USA). Differences between treatments were compared using the Duncan test at the 95% confidence level. Non-linear regressions between g_s and Ψl, g_s and Ψs, A_N and g_s, E and g_s, E and Ψs, and A_N g_s^{-1} and g_s were developed for different levels of vine water status by each grapevine variety. The coefficient of determination (r^2) was used to evaluate how well the regression line represents the data. The relationship between net CO_2 assimilation (A_N) and stomatal conductance (g_s) in each variety was contrasted with that in the literature in order to compare how these varieties behave under different experimental conditions.

RESULTS AND DISCUSSION

Relationship between stomatal conductance and plant water status

To evaluate genotypic sensitivity to water deficit, the stomatal conductance (g_s) was compared with the leaf water potential (Ψl) and stem water potential (Ψs) (Figs 1 and 2 respectively) in the 2011/2012 and 2012/2013 seasons. For similar values of Ψl and Ψs, all varieties except Sauvignon blanc presented higher maximum values of g_s during the first season (2011/2012) than during the second season (2012/2013) (Figs 1 and 2), as well as lower minimum g_s values (Figs 1 and 2). During the 2011/2012 season, minimum g_s values for Chardonnay, Merlot, Pinot noir and Sauvignon blanc were 0.02, 0.01, 0.05 and 0.03 mol H_2O m^{-2} s^{-1} respectively, whereas they were 0.05, 0.05, 0.11 and 0.08 mol H_2O m^{-2} s^{-1} respectively in the 2012/2013 season. This higher sensitivity of the stomatal response to water deficit during the former season is probably related to the higher vapour-pressure deficit (VPD) experienced at the time of the measurements, reaching 1.05 kPa, compared to the 0.95 kPa reached during the second season. Prieto *et al*. (2010) reported that Syrah, Marselan, Mouvèdre and Ekigaïna grapevine varieties presented higher stomatal regulation in response to increased ambient VPD. Moreover, even under mild to moderate water stress (-0.9 ≤ Ψl ≤ -1.3 MPa), the variability of g_s values was also higher in 2011/2012 than in 2012/2013 (Fig. 1). Generally, when Ψl was used as an indicator of water status, higher g_s magnitudes and more variability were observed at lower water potentials than when Ψs was used. Although Ψl varies according to vine water status, it is also dependent on the microclimatic environment of the leaf; due to this, midday Ψl probably is not a very accurate indicator of vine water status (Van Leeuwen *et al*., 2007). Moreover, a clear separation in g_s magnitudes between stressed and non-stressed grapevines was only found when water status was characterised as Ψs (Fig. 2). The variability in g_s decreased in most of the grapevine varieties in the 2012/2013 season, especially for Ψs values lower than -0.9 MPa (moderate water deficit) (Fig. 2).

Regarding g_s sensitivity, Chardonnay and Merlot reached maximum g_s values of 0.45 mol H_2O m^{-2} s^{-1}, and minimum g_s values of 0.02 mol H_2O m^{-2} s^{-1} and 0.01 mol H_2O m^{-2} s^{-1} respectively, while Pinot noir and Sauvignon blanc reached maximum g_s values of 0.63 mol H_2O m^{-2} s^{-1}, and minimum g_s values of 0.05 mol H_2O m^{-2} s^{-1} and 0.03 mol H_2O m^{-2} s^{-1} respectively. Stomatal conductance is not only related to the availability of soil water and VPD, but also the interactions of internal and external leaf factors, such as hydraulic adjustment, root signals or anatomical architecture (Collins *et al*., 2010; Pou *et al*., 2012; Gerzon *et al*., 2015). Indeed, it has been shown that differences in the abscisic acid concentration [ABA] in the xylem sap may explain the more sensitive reaction to water deficit in isohydric grapevine varieties compared to anisohydric varieties (Chaves *et al*., 2016). ABA could be involved in the closure of aquaporins in bundle sheath cells, decreasing the water flow to the mesophyll cells and strengthening the implications of a hydraulic component in stomata closure (Chaves *et al*., 2016). Thus, according to the results presented in Figs 1 and 2, this might be the case in Sauvignon blanc, since there is rapid stomatal closure as the water content in the soil decreases. In this way, the leaf water status interacts strongly with gas exchange and, consequently, there is a well-defined correlation between Ψl and g_s (Prieto *et al*., 2010).

The Merlot, Pinot noir and Chardonnay grapevines progressively decreased their g_s as stem water potential (Ψs) became more negative due to high VPD and conditions of water stress. These varieties maintained moderately high g_s levels under mild water deficit conditions (-0.9 MPa ≤ Ψs ≤ -0.6 MPa), as defined by Van Leeuwen *et al*. (2009). However, g_s progressively declined with mild to moderate water stress (Ψs < -0.6 MPa), and remained constant at a g_s value close to 0.1 mol H_2O m^{-2} s^{-1}. This physiological behaviour is common

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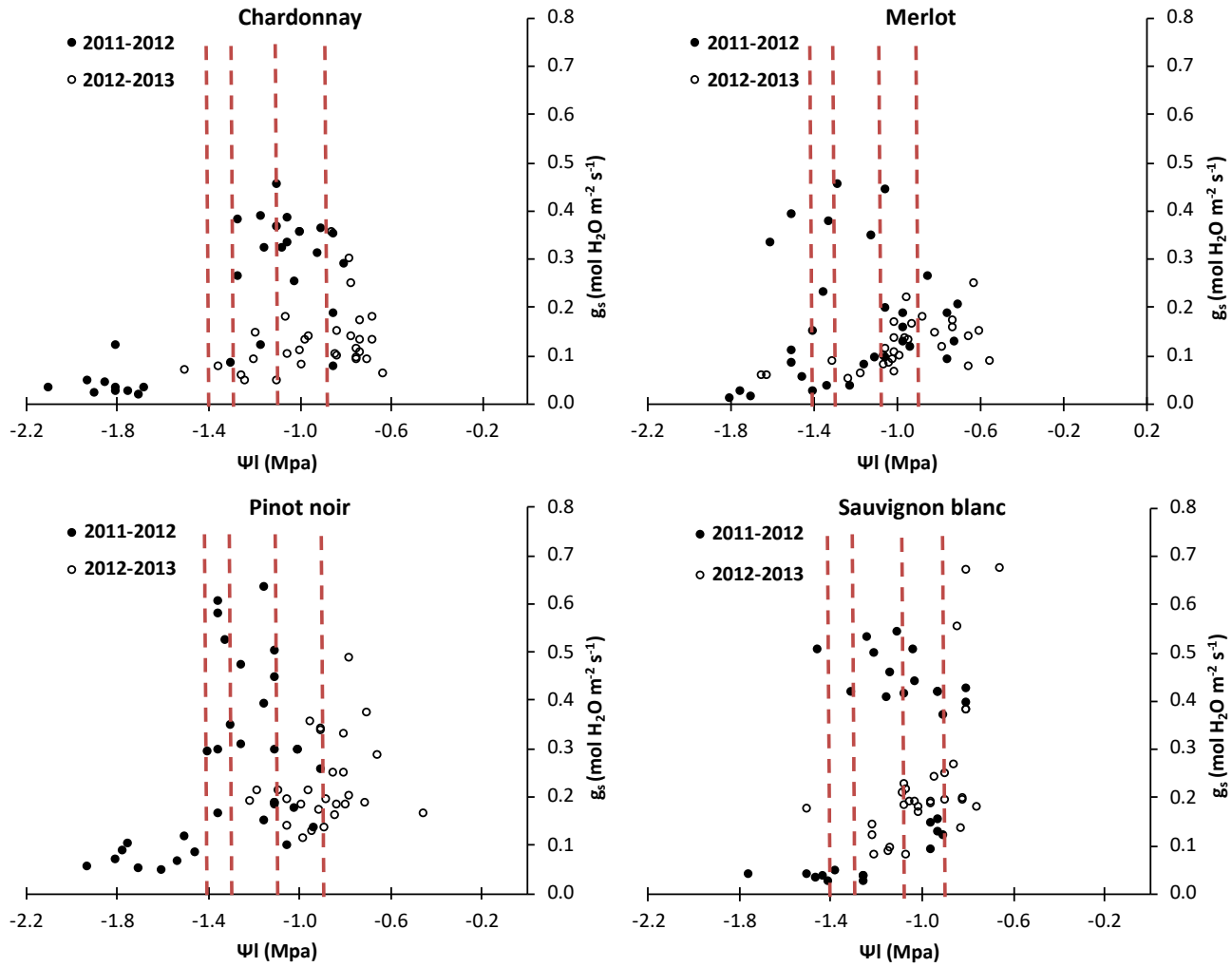

FIGURE 1

Relationship between stomatal conductance (g_s) and leaf water potential (Ψl) in the 2011/2012 (black circles) and 2012/2013 (white circles) seasons. Values were divided into five ranges according to the thresholds proposed by Van Leeuwen *et al*. (2009): i) no water deficit (***Ψl*** > -0.9 MPa); ii) mild water deficit (-0.9 ≤ ***Ψl*** ≤ -1.1 MPa); iii) moderate to mild water deficit (-1.1 ≤ ***Ψl*** ≤ -1.3 MPa); iv) moderate to severe water deficit (-1.3 ≤ ***Ψl*** ≤ -1.4 MPa); and v) severe water deficit (***Ψl*** < -1.4 MPa).

in species described as having anisohydric responses to soil water deficit (Pou *et al*., 2012). Thus, compared to isohydric behaviour, anisohydric behaviour involves the consumption of soil water resources by roots until lower water potentials are achieved (Chaves *et al*., 2016). Merlot and Chardonnay presented lower Ψs values than Pinot noir and Sauvignon blanc (Fig. 2). In contrast, Sauvignon blanc grapevines managed under conditions of no water deficit (Ψs ≥ -0.6 MPa). They presented high g_s values (≥ 0.4 mol H_2O m^{-2} s^{-1}) and, after this point, the values dropped drastically (0.25 ≤ mol H_2O m^{-2} s^{-1}) during the first season, leading to decreased transpiration and, consequently, decreased diffusion of CO_2 into the plant. During the second season, Pinot noir and Sauvignon blanc decreased their g_s down to 0.2 mol H_2O m^{-2} s^{-1} at Ψs values of -0.3 MPa (no water stress) (Fig. 2), thus avoiding a drastic fall in Ψs. Pinor noir showed different hydric behaviour in the two experimental seasons. As a consequence, this variety was described as displaying anisohydric behaviour in the first season and isohydric behaviour in the second.

Relationship between net CO_2 assimilation and stomatal conductance

A typical exponential relationship for net CO_2 assimilation (A_N) and stomatal conductance (g_s) was found on the basis of the data measured during the field trial. Our data are located around the curve proposed by Medrano *et al*. (2002), which is considered representative for most grapevine varieties (Fig. 3). Moreover, different relationships between A_N and g_s for each variety were obtained in both experimental years (Fig. 4).

The obtained A_N data was homogeneously distributed throughout the g_s data spectra for the Chardonnay, Pinot noir and Merlot grapevines (Fig. 4). Thus, as the g_s increased, there was greater A_N. In contrast, there were no A_N values in Sauvignon blanc when the g_s values were between 0.25 and 0.4 mol H_2O m^{-2} s^{-1}. At one extreme, when well-watered conditions were applied, this variety

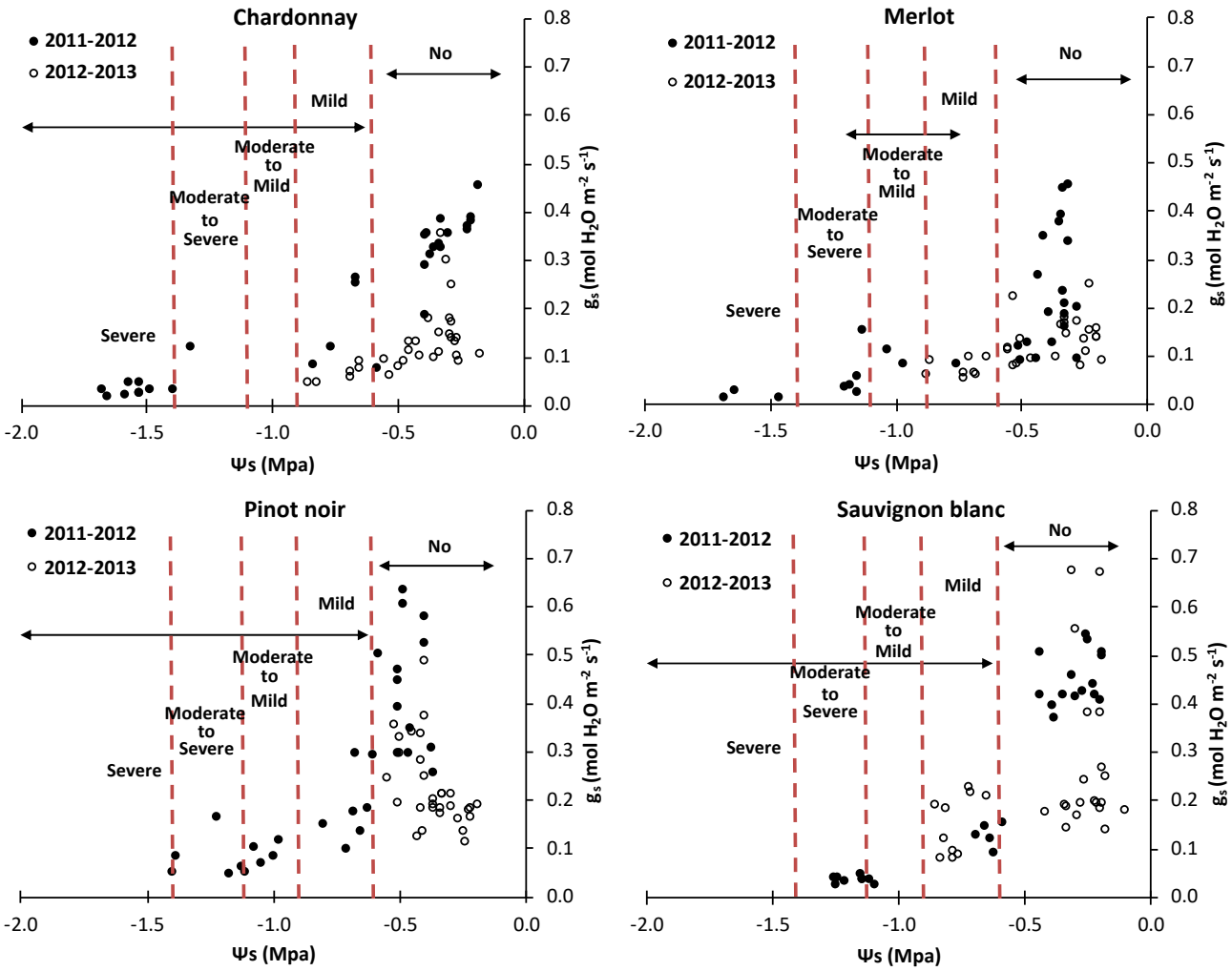

FIGURE 2

Relationship between stomatal conductance (g_s) and stem water potential (Ψs) in the 2011/2012 (black circles) and 2012/2013 (white circles) seasons. Values were divided into five ranges according to the thresholds proposed by Van Leeuwen *et al*. (2009): i) no water deficit (***Ψ***s > -0.6 MPa); ii) mild water deficit (-0.6 ≤ ***Ψ***s ≤ -0.9 MPa); iii) moderate to mild water deficit (-0.9 ≤ ***Ψ***s ≤ -1.1 MPa); iv) moderate to severe water deficit (-1.1 ≤ ***Ψ***s ≤ -1.4 MPa); and v) severe water deficit (***Ψ***s < -1.4 MPa).

assimilated CO_2 at a high rate. However, under water-stressed conditions, g_s fell considerably in comparison to the rest of the grapevine varieties, probably due its strong stomatal regulation, leading to a fast decrease in A_N. This protective physiological response might have costs in terms of lower CO_2 assimilation rates during water stress, leading to a reduction of growth, and under severe abiotic stress this could reach a critical threshold for leaf damage (Chaves *et al*., 2010, 2016; Pou *et al*., 2012). One the other hand, Pinot noir grapevines growing under severe water-stress conditions did not completely close their stomata, maintaining a higher CO_2 assimilation rate than the other studied varieties. This could imply that, in this variety, lower g_s magnitudes could be less limiting for CO_2 assimilation than for the transpiration rate compared to other varieties. Additionally, under well-watered conditions, this grapevine variety presented the highest maximum A_N value compared with the rest of the studied varieties (Fig. 4), suggesting near-anisohydric behaviour in this case. Accordingly, Chaves *et al*. (2016) report that anisohydric varieties present cooler leaves and higher photosynthetic rates than isohydric ones. However, anisohydric grapevines may suffer accelerated dehydration under severe drought stress due to their high transpiration rates, which are not compensated by soil water uptake (Chaves *et al*., 2016). With respect to stomatal control strategy, certain authors have reported that Pinot noir behaved as an anisohydric variety when water stress was applied at the pre-véraison stage and as an isohydric variety when it was applied at the post-véraison stage (Poni *et al*., 1993; Lovisolo *et al*., 2010).

Transpiration according to stomatal conductance and plant water status

Grapevines close their stomata under conditions of water stress, leading to a decrease in transpiration (E). Therefore, g_s has a great influence on E (Fig. 5). Correlation coefficients (r^2) between E and g_s were higher than 0.83 for the 2011/2012 season (data not shown). However, the obtained r^2 in the

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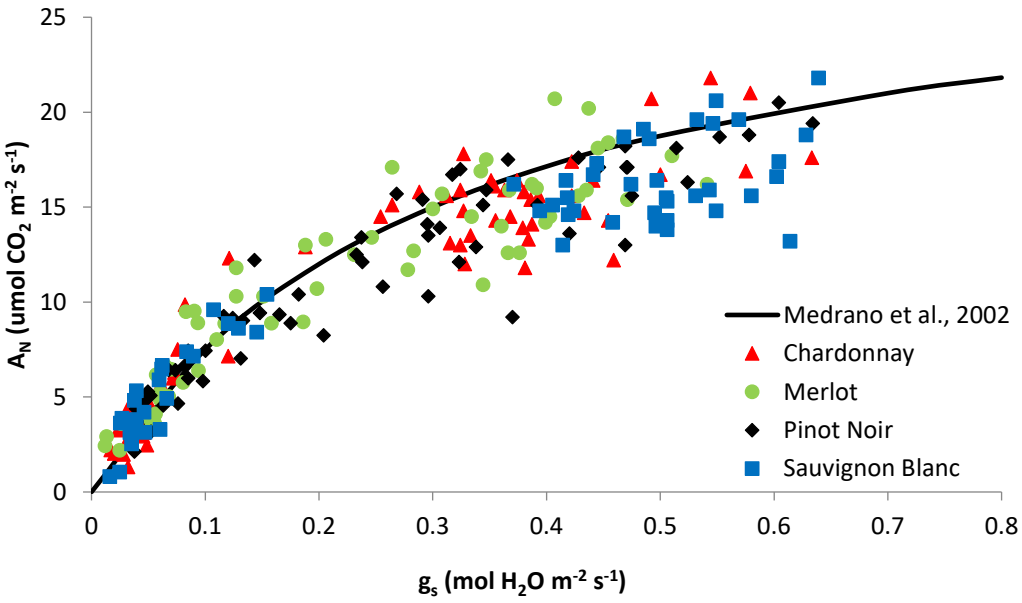

FIGURE 3
Relationship between net CO_2 assimilation (A_N) and stomatal conductance (g_s) measured in the 2011/2012 season compared to the data observed by Medrano *et al*. (2002).

2012/2013 season were only significant for Chardonnay (r^2 = 0.71). This was probably because the treatments performed during the second season did not generate a severe water deficit, which mean there were no extreme values.

The 2011/2012 season was warmer than the 2012/2013 season and presented a higher reference evapotranspiration (ET_o) and vapour-pressure deficit (VPD). This had a direct effect on E and stomatal conductance (g_s) rates, which were greater in the 2011/2012 season, favouring more negative water potentials.

E declined as Ψs became more negative. Similar E magnitudes close to 12 mmol H_2O m^{-2} s^{-1} were observed in Chardonnay, Merlot and Sauvignon blanc grapevines for the 2011/2012 season (Fig. 6). Pinot noir grapevines reached maximum E values of slightly below the others. Chardonnay grapevines presented a progressive drop in E from 10.3 to 1.1 mmol H_2O m^{-2} s^{-1}, and Ψs values lower than -0.2 MPa (non-stressed vines). This behaviour was similar to that found in Merlot, Pinot noir and Sauvignon blanc grapevines, which presented a progressive fall in E as Ψs became more negative. In Chardonnay and Merlot, levels of Ψs below -1.6 MPa (severe water stress) gave rise to minimum E (< 1.1 mmol H_2O m^{-2} s^{-1}). However, the minimum value of E (1.45 mmol H_2O m^{-2} s^{-1}) in Sauvignon blanc was reached at -1.1 MPa, defined as moderate to severe water stress by Van Leeuwen *et al*. (2009). In addition, high levels of E were reached under non-stress conditions in Sauvignon blanc grapevines, and subsequently E fell drastically to Ψs values lower than -0.6 MPa, with an average value of 3.1 mmol H_2O m^{-2} s^{-1}. Moreover, stressed Pinot noir grapevines presented higher levels of E at very negative Ψs (~ -1.4 MPa) than those obtained in Sauvignon blanc, Chardonnay and Merlot. In Pinot noir, E fluctuated slightly at Ψs values lower than -0.9 MPa, ranging from 4.8 to 2.3 mmol H_2O m^{-2} s^{-1}, with a minimum value of 2.33 mmol H_2O m^{-2} s^{-1} at -1.2 MPa. Then, under moderate to severe water-stress conditions, E fluctuated between 1.45 and 2.26 mmol H_2O m^{-2} s^{-1}. Therefore, E followed the same behaviour as g_s and contributed to the explanation of the isohydric (Sauvignon blanc) or anisohydric (Merlot and Chardonnay) behaviour of the varieties.

Intrinsic water-use efficiency

Merlot and Chardonnay grapevines showed higher levels of A_N g_s^{-1} than Pinot noir, mainly due to the low levels of g_s obtained; therefore, it is likely that these varieties partially owe their higher efficiency in the use of water to the fact that their stomata were not completely open (Table 1). Sauvignon blanc and Pinot noir reached a lower A_N g_s^{-1} than Merlot, together with high levels of g_s, which could affect their productive potential under conditions of unfavourable water availability. Based on the literature, isohydric varieties under high evaporative demand have been considered as experiencing a more pronounced increase in A_N g_s^{-1}, thus being better adapted to drought stress than anisohydric varieties (Schultz 2003; Vandeleur *et al*., 2009). However, other reports in the literature show that the same variety could behave as iso- or anisohydric, depending on the experimental conditions (Chaves *et al*., 2010; Lovisolo *et al*., 2010). Moreover, Pou *et al*. (2012) showed that Chardonnay (considered an anisohydric variety) displayed higher water-use efficiency at the leaf level than two other isohydric varieties.

A_N g_s^{-1} is independent of atmospheric conditions, since it measures the ability of the leaf to regulate photosynthesis and g_s (Medrano *et al*., 2007). Fig. 7 shows the different

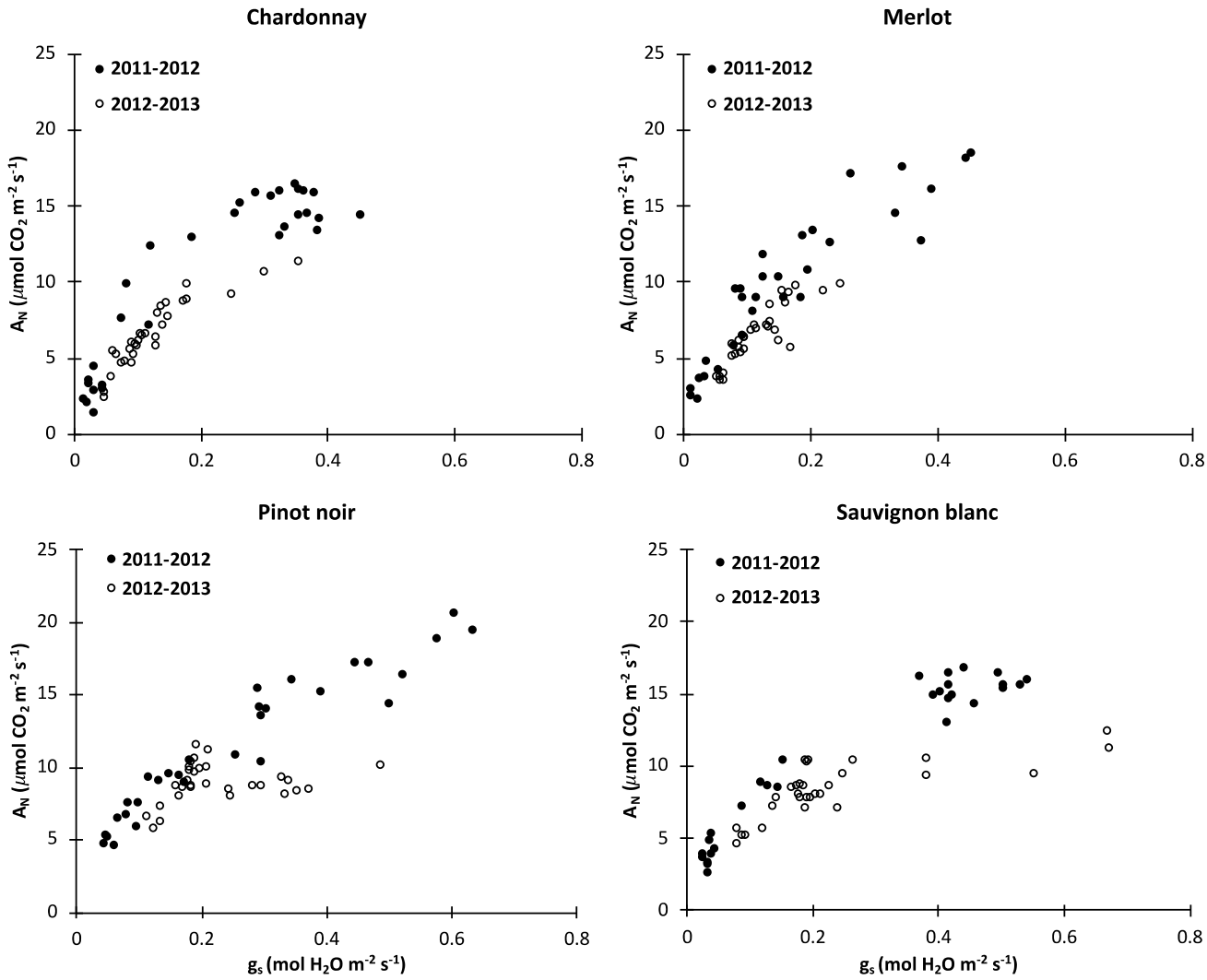

FIGURE 4
Relationship between net CO_2 assimilation (A_N) and stomatal conductance (g_s) in the 2011/2012 (black circles) and 2012/2013 (white circles) seasons.

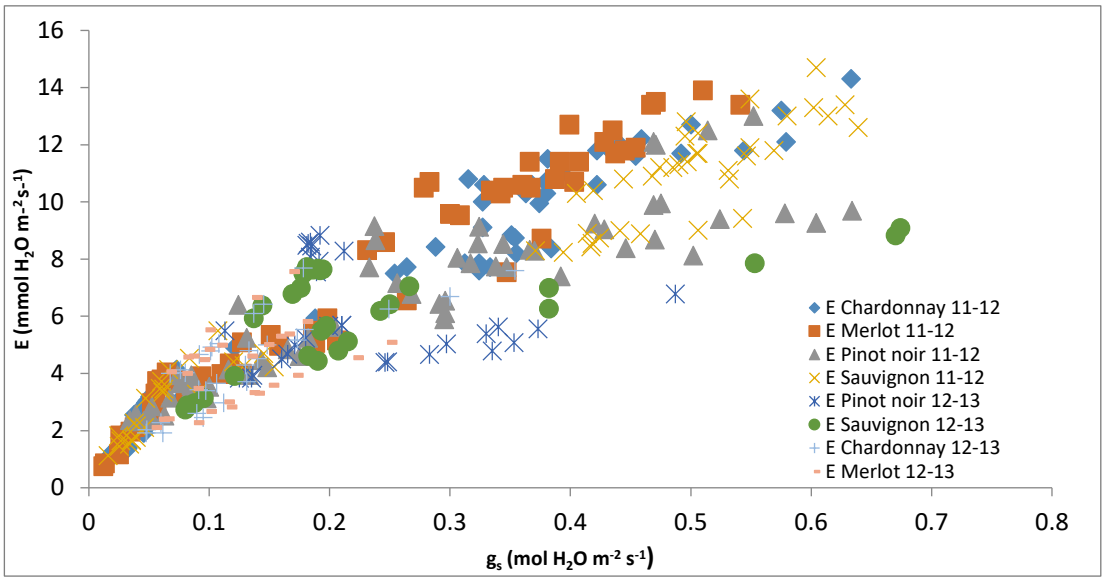

FIGURE 5
Relationship between transpiration (E) and stomatal conductance (g_s) during the 2011/2012 and 2012/2013 seasons.

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values of g_s measured during the first and second experimental seasons and their respective estimated water-use efficiency (WUE) values. Based on our results for g_s values between 0.70 and 0.14 mol H_2O m^{-2} s^{-1} (slight stress) with adequate water availability, A_N g_s^{-1} gradually increased as the water status of the grapevines decreased. Under these conditions, A_N decreased slightly and stomatal closure limited photosynthesis. Thereafter, as g_s decreased from 0.14 mol H_2O m^{-2} s^{-1} to 0.05 mol H_2O m^{-2} s^{-1} (moderate water stress), A_N decreased and A_N g_s^{-1} increased significantly. Under these conditions, stomatal limitations dominated and photosynthesis was reversible. Finally, at g_s levels lower than

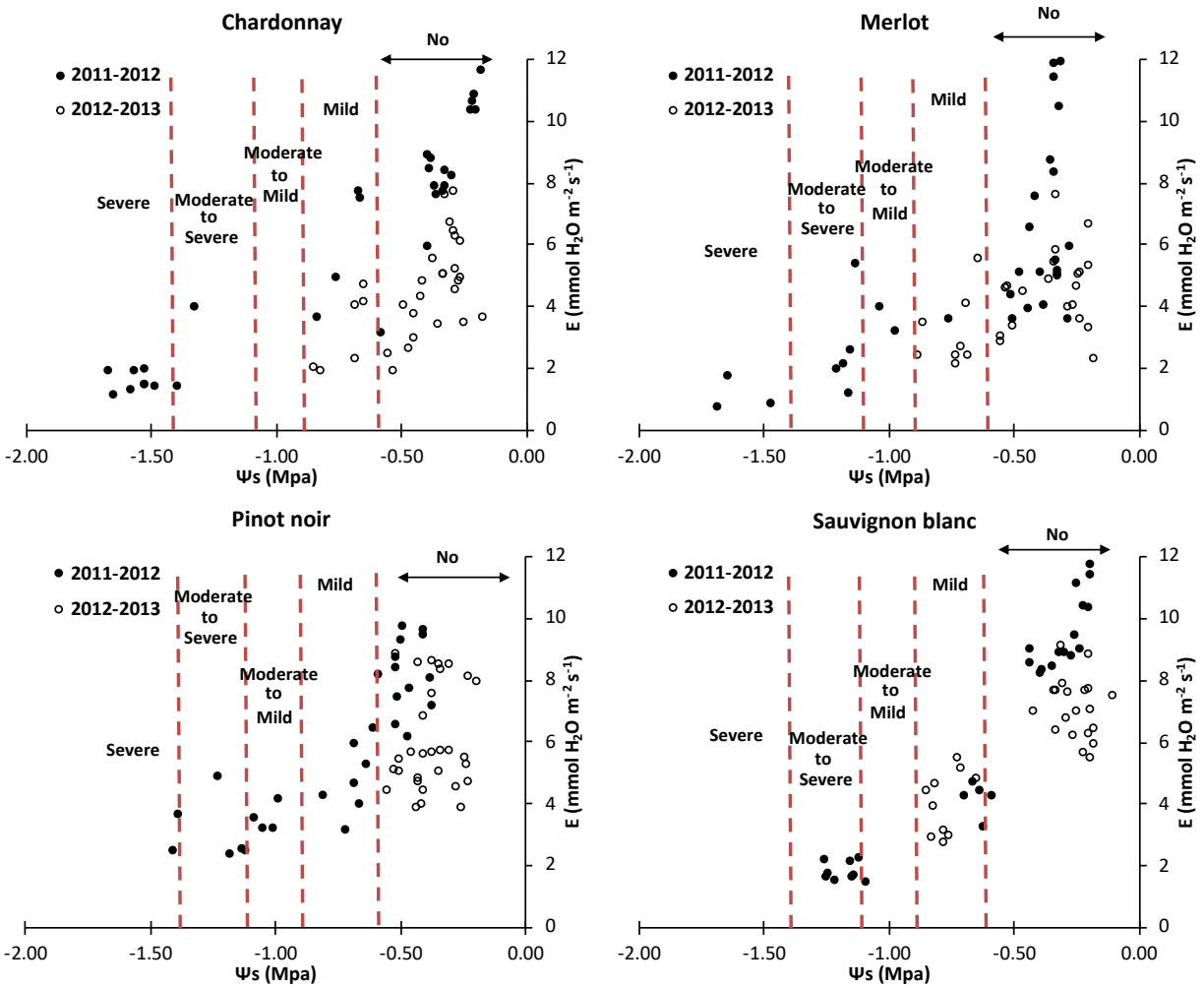

FIGURE 6

Relationship between transpiration (E) and stem water potential (Ψs) during the 2011/2012 (black circles) and 2012/2013 (white circles) seasons. Values were divided into five ranges according to the thresholds proposed by Van Leeuwen *et al*. (2009): i) no water deficit (***Ψs*** > -0.6 MPa); ii) mild water deficit (-0.6 ≤ ***Ψs*** ≤ -0.9 MPa); iii) moderate to mild water deficit (-0.9 ≤ ***Ψs*** ≤ -1.1 MPa); iv) moderate to severe water deficit (-1.1 ≤ ***Ψs*** ≤ -1.4 MPa); and v) severe water deficit (***Ψs*** < -1.4 MPa).

TABLE 1

Mean stomatal conductance (g_s), net CO_2 assimilation (A_N) and intrinsic water-use efficiency (A_N g_s^{-1}) over the 2011/2012 and 2012/2013 seasons for each grapevine variety.

	g_s (mol H_2O m^{-2} s^{-1})	A_N (µmol CO_2 m^{-2} s^{-1})	A_N/g_s (µmol CO_2 mol H_2O^{-1})
Pinot noir	0.24 [b]	10.12 [b]	50.51 [a]
Sauvignon blanc	0.25 [b]	9.43 [ab]	53.08 [ab]
Chardonnay	0.17 [a]	8.50 [a]	62.25 [bc]
Merlot	0.14 [a]	8.16 [a]	69.74 [c]

For each parameter (n = 20), different letters in the same row indicate significant differences between treatments (Duncan test, $p \leq 0.05$).

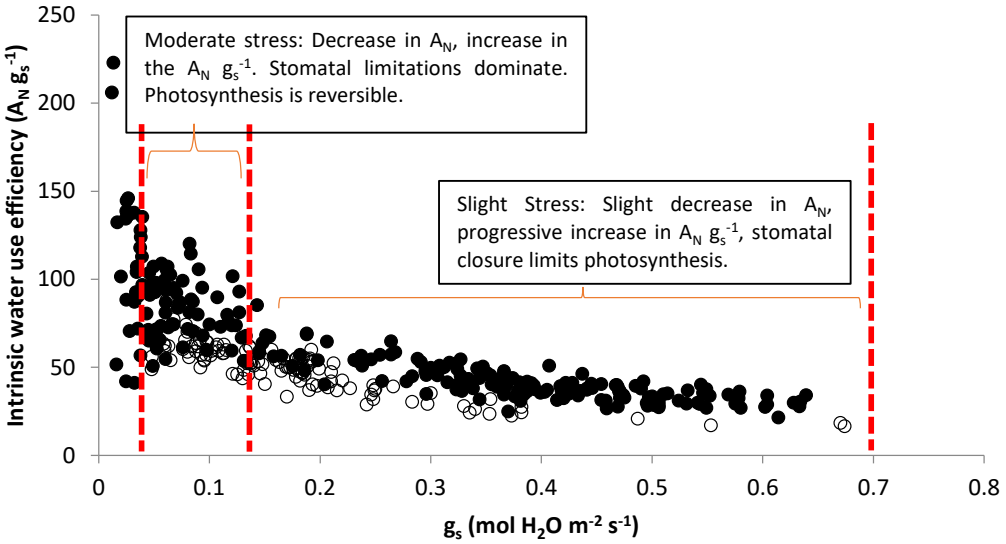

FIGURE 7

Relationship between intrinsic water-use efficiency (A_N g_s^{-1}) and stomatal conductance (g_s) in the 2011/2012 (black circles) and 2012/2013 (white circles) seasons. The three delimited regions in the figure follow the g_s thresholds proposed by Medrano *et al*. (2002).

0.05 mol H_2O m^{-2} s^{-1} (severe water stress), A_N g_s^{-1} decreased drastically again, as has been reported by Cifre *et al*. (2005). Therefore, grapevines become less efficient in the use of water when there is severe water stress represented by low g_s levels (Fig. 7). The depicted results displayed under moderate water stress differ from those reported by Douthe *et al*. (2018) when measuring WUE at the whole-plant scale. These authors showed that carbon and water fluxes were drastically reduced, while estimated WUE was not improved but decreased. So, in this case, scaling up WUE readings from leaves to the whole plant leads to some discrepancies among single-leaf and whole-canopy results. However, the results reported by these authors are in agreement with those shown in Fig. 7 for grapevines growing under severe water stress. Grapevine leaves from all locations in the canopy, except those located in the central part, showed a similar radiation-use efficiency, suggesting that light interception considerably affects variations in photosynthesis within the grapevine canopy (Escalona *et al*., 2003). Thus, it is possible that, under severe water stress, variations between leaves within the canopy disappear and any single leaf may reflect what is happening at the whole-plant level. Additionally, other factors, such as nocturnal water loss, changes in dry matter, partitioning among the sinks, or harvest load respiration rates, could also explain the lack of correlation usually reported between instantaneous WUE and intrinsic water-use efficiency (WUEi) (Douthe *et al*., 2018).

CONCLUSIONS

Chardonnay and Merlot decreased their leaf and stem water potential in accordance with the applied water stress and reached high levels of intrinsic water-use efficiency (WUEi) (A_N g_s^{-1}) (62.25 and 69.74 ***µmol*** CO_2 mol H_2O^{-1} respectively). Thereby, Chardonnay and Merlot could be characterised in this trial as anisohydric varieties. In contrast, Sauvignon blanc drastically decreased g_s (0.67 to 0.03 mol H_2O m^{-2} s^{-1}) and E (11.7 to 1.45 mmol H_2O m^{-2} s^{-1}) under conditions of water stress, leading to a strong decrease in A_N (2.53 ***µmol*** CO_2 m^{-2} s^{-1}), thus displaying isohydric behaviour. In Pinot noir, g_s dropped (0.63 to 0.05 mol H_2O m^{-2} s^{-1}) in relation to the water deficit, and Ψl changed in association with the irrigation treatments, probably due to the availability of water in the soil. However, under stress conditions, A_N g_s^{-1} was lower in this variety (50.51 ***µmol*** CO_2 mol H_2O^{-1}). Accordingly, this variety could be characterised as either an isohydric or anisohydric variety. Consequently, these results suggest that Pinot noir may be considered as a near-anisohydric variety. In general, physiological responses of the varieties are directly related to the climate and water content in the soil and may vary from one area to another. For this reason, other physiological parameters could be measured to characterise more accurately the isohydric or anisohydric behaviour of the varieties of this study.

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