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Soil water-holding capacity mediates hydraulic and hormonal signals of near-isohydric and near-anisohydric *Vitis* cultivars in potted grapevines.

Abridged title: Soil and genotype influence on grapevine response to drought.

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Summary Text for the Table of Contents.

The ecophysiological behaviour of grapevine cultivars in response to drought is influenced by the soil conditions and by the plant genotype. These two components interact through a complex of hydraulic and hormonal signal exchanges occurring between roots and leaves. Our work highlighs the differences in these signals observed in a near-isohydric and a near-anisohydric grapevine cultivars on two soil substrates with different textures, causing different dynamics of water deprivation during an imposed increasing water stress.

Abstract

Grapevine (*Vitis vinifera* L.) expresses different responses to water stress, not only depending from genotype, but also from the influence of vineyard growing conditions or seasonality. We aimed to analyze the effects on drought response of two grapevine cultivars growing on two soils, one water draining (WD) containing sand 80% vol. and the other water retaining (WR), with no sand. Under these two different water-holding capacities Syrah, displaying a near-anisohydric response to water stress, and Cabernet

Sauvignon (on the contrary, near-isohydric) were submitted to water stress in a pot trial. Xylem embolism contributed to plant adaptation to soil water deprivation: in both cultivars during late phases of water stress, however, in Syrah, already at moderate early stress levels. By contrast, Syrah showed a less effective stomatal control of drought than Cabernet Sauvignon. The abscisic acid (ABA) influenced tightly the stomatal conductance of Cabernet Sauvignon on both pot soils. In the near-anisohydric variety Syrah an ABA-related stomatal closure was induced in WR soil to maintain high levels of water potential, showing that a soil-related hormonal root-to-shoot signal causing stomatal closure superimposes on the putatively variety-induced anisohydric response to water stress.

Key words: abscisic acid (ABA), cavitation, embolism, hydraulic conductance, water potential.

Introduction

Grapevine (*Vitis vinifera* L.) is a species expressing both isohydric and anisohydric behaviours, not only depending from genotype (Schultz 2003), but also from the influence of growing conditions or seasonality (Chaves *et al.* 2010, de Souza *et al*. 2003) or from the environmental conditions to which the plant was exposed (Collins *et al.* 2010; Lovisolo *et al.* 2010; Pou *et al.* 2012; Tramontini *et al.* 2013*a*).

Although the genotype itself is not sufficient to preview the physiological behaviour of grapevine plants, some cultivars have been more frequently observed expressing consistent results than others. One of these is Syrah. This cultivar, of mesic origin, has been mainly categorized as anisohydric, either from observations of plants under field conditions (Schultz 2003; Rogiers *et al.* 2009; Soar *et al.* 2009) or in pots (Soar *et al.* 2006). Cabernet Sauvignon, on the other hand, has been more frequently observed to display a response to water deprivation nearer to isohydric type (Hochberg *et al.* 2013). Owing to the differential response observed on these two cultivars under the same water conditions, Cabernet Sauvignon and Syrah have already been coupled in comparative experiments (Chalmers 2007; Petrie and Sadras 2008; Rogiers *et al.* 2009; Hochberg *et al.* 2013) and can therefore be selected as efficient models for representing iso- and anisohydric behaviours.

The stomatal control, which is an endogenous, but highly variable character, was considered in combination with the soil effect. Soil is in fact another crucial component in grape and wine production, not only because it determines the water and nutrients availability for the plant and therefore its productive performances, but also for its specific implication in the "*terroir* effect" in viticulture (Bodin and Morlat 2006; van Leeuwen *et al.* 2009). In spite of the acknowledged importance on grape and wine production, not many studies attempted to quantify its effects with comparative trials. For this reason, in the presented work, we decided to focus the attention only on the differences produced by two soils in terms of soil texture and related water availability provided to the plant: one single aspect which is, however, strongly influenced by physical, chemical, and biological properties of the substrate. When a soil dries, in fact, the increasing drought affects the plant in multiple and complex ways (Whitmore and Whalley 2009).

Cavitation of the xylem vessels is a very relevant consequence of the limited soil moisture, as it can produce dramatic consequences by reducing the hydraulic conductivity of the vascular tissues and impairing the possibility for the plant to replace transpired water (Brodersen *et al.* 2013). It is also one of the most studied effects of drought in grapevine, in combination with loss in hydraulic conductance (Lovisolo and Tramontini 2010). In leaves, cavitation and consequent embolism formation affect mainly the leaf midrib (Blackman *et al.* 2010), with a conductivity loss in grapevine petioles of 50% at Ψ_{stem} of -0.95 MPa and of more than 90% at -1.5MPa (Zufferey *et al.* 2011). On the other hand, the entity of damage produced by cavitation and the break against its propagation are modulated by the speed and intensity of stomata reaction and by its effect on transpiration (Domec and Johnson 2012) approximating leaves to hydraulic fuses of the plant (Zufferey *et al.* 2011).

Embolism formation and repair is controlled by a likely hydraulic mediation at the leaf level (Pantin *et al*. 2013) and via chemical signals (Salleo *et al.* 1996; Lovisolo and Schubert 2006) among which abscisic acid (ABA) has a crucial role. ABA is in fact the hormone devoted to drive the stomatal response to drought: when the soil water potential declines, ABA acts as a messenger indicating water stress from the roots, via the xylem sap, to the guard cells in the leaves and inducing the stomata closure (Hartung *et al.* 2002), limiting in such a way the potential consequences of embolism formation (Chitarra et al. 2014). When the water availability is recovered to an adequate level, the roots stop releasing the hormone and the stomata re-open. The delayed interruption of the signal, much more gradual than the initial release, suggests a further action of the hormone on the embolisms repair (Lovisolo *et al.* 2008; Perrone *et al.* 2012).

Furthermore, in grapevine metabolic and hydraulic behaviour have shown to be related, according to the observations recently published by Hochberg *et al.* (2013) from a study conducted on Cabernet Sauvignon and Syrah plants too. In this work the more anisohydric grapevine cultivar showed higher water uptake and higher g_s than the near-isohydric cultivar.

The aim of the present work is to analyze the effect of two types of drying soil, differing in water retaining properties, on two grapevines genotypes, characterized by different ecophysiological behaviour, from the point of view of the hydraulic balance of the plant (i.e. water potential, stomatal control, embolism formation), and its hormonal(ABA) control of water losses.

Materials and Methods

Plant material and growing conditions

The trial was conducted in August 2012 at Hochschule Geisenheim University (Geisenheim, Germany) on 16 three-year-old plants of *Vitis vinifera* L. of two genotypes: 8 plants of 'Cabernet Sauvignon' and 8 of 'Syrah'. Both were grafted on hybrids of *Vitis berlandieri* × *Vitis riparia* ('161-49 Couderc'for 'Cabernet Sauvignon' and '420A Millardet Et De Grasset' for 'Syrah') of comparable characteristics (Whiting 2004), especially in controlling the interrelationship between leaf or stem water potential and stomatal conductance (Tramontini *et al*. 2013b). The plants were maintained under glasshouse conditions with no supplementary light or heating in 9 L (24 cm average diameter) plastic pots filled (20 cm depth) with two different substrates, one water draining (WD soil) and the other water retaining (WR soil). The WD substrate was composed of 80 % vol. of sand and 20 % vol. of ED 73 (Einheitserde Classic, Einheitserde-Einheitserde- und Humuswerke Gebr. Patzer GmbH & Co.KG, Sinntal, Germany; consisting of 55% white peat, 30% clay, 15% sod peat; chemical properties pH ($CaCl_2$) 5.8, salt content 2.5 g L^{-1}) including nutrient salt (14+16+18, 1 kg m^{-3}) and a slow-release fertilizer (Gepac LZD 20+10+15, 2 kg m^{-3}), the WR substrate consisted entirely of ED 73.

Plants were watered to container capacity at the beginning of the experiment (Tramontini *et al*. 2013b) and fertilized in order to bring them to the same level of nitrogen availability. Soil nitrogen content after the fertilization was estimated according to Robinson recommendations (1988), confirming that at the beginning of the experiment the two different substrates had approximately the same amount of available nitrogen. Data collection started when the plants had reached a mild water stress (Ψ_{stem} ≤ -0.5 MPa), such as four days after interruption of irrigation. In that moment plants had 14.4 ± 2.8 leaves with no significant differences between cultivars or soils. Each plant was excluded from the trial when wilting was observed.

Soil water content (θ, %), soil water potential (Ψ_{soil}, MPa), stem water potential (Ψ_{stem}, MPa), xylem embolism extent and stomatal conductance (g_s, mmol m^{-2} s^{-1}) were assessed during the whole duration of the experiment. All measurements were taken daily between 9:30-12:00 and 14:00-17:00 in order to standardize putative control of circadian expression in cell water channels (Uehlein and Kaldenhoff 2006).

Water relations

Soil water content (θ) was gravimetrically determined by collecting daily approximately 10 ml of soil from three different points and depths in each pot (5, 10, 15 cm depth at the half of rays 120° distant one from the other). The soil was weighed, oven-dried at 100 °C for 24 h and then re-weighed to assess water content. At the same time, the water retention curves for the two soils were assessed with pressure plate measurements of the potting substrate (Richards 1965), obtaining two equations:

WR soil $-\Psi_{soil} = 53.791 * e^{-0.127 * \theta}$

WD soil $-\Psi_{soil} = 1.3423 * e^{-0.264 * \theta}$

The obtained relationships allowed for the calculation of Ψ_{soil} based on θ.

Ψ_{stem} was measured on mature, undamaged and non-senescent leaves using a pressure chamber (Soilmoisture Corp., Santa Barbara, CA, USA) (Scholander *et al.* 1965) at midday according to Turner (1988). Prior to the measurements leaves were bagged with a plastic sheet and covered with aluminium foil to stop transpiration at least 1 h before measurements were taken.

Xylem embolism

Daily determination of xylem embolisms in leaf petioles, induced by the presence of air bubbles in xylem vessels, was carried out around midday using a high-pressure flowmeter (HPFM, Dynamax Inc., Houston, TX, USA) (Tyree *et al.* 1995). As the assessment of embolism extent is a destructive analysis, leaf petioles were used as a proxy of the plant behaviour (Lovisolo *et al.* 2008; Perrone *et al.* 2012). During the whole duration of the experiment macro- and microbubbles were regularly flushed out of the system according to the manufacturer`s instruction manual and the mismatch between the two pressure transducers was controlled daily by running the ‘Set Zero’ routine before measuring.

For each determination of percent loss of conductivity (PLC), the petioles and leaves were cut under water from the shoots and immediately attached to the HPFM tubing under water preventing air bubbles to enter the system. The leaves were cut ~1 cm above the petiole insertion a few seconds after starting the measurement. The initial hydraulic conductance K_{hi} was determined applying an initial pressure of ~20 kPa for 3 min. Distilled and degassed water with an addition of 10 mmol L^{-1} KCl was used as perfusion liquid. Petioles were then flushed for 3 min applying a transient increase of pressure until a pressure of ~550 kPa was reached. This pressure was kept constant for 3 min. To determine the final hydraulic conductance K_{hf} the pressure was downregulated to ~20 kPa and held constant for 3 min. To calculate K_{hi} and K_{hf} average values of the hydraulic conductance of the respective timespans were used.

Data were displayed and stored using the software HPFM95-XP Version 1.12 (Dynamax Inc.) and exported and processed using Microsoft Excel.

The percent loss of conductivity (PLC) was determined as follows:

$$\mathrm{PLC}\ [\%] = \frac{(K_{hf} - K_{hi})}{K_{hf}} * 100$$

After the embolism determination the length and the maximum and minimum diameter of the petioles was assessed.

Stomatal conductance

Measurements of g_s were carried out on adult, non-senescent leaves that were well-exposed to direct sunlight. G_s was measured using a porometer (AP4, Delta-T Devices Ltd, Cambridge, UK). Measurements on three leaves per plant were taken for every measuring cycle and the g_s values of the three leaves were averaged.

Analysis of abscisic acid (ABA) in leaves

ABA was extracted from leaves where stomatal conductance was assessed applying the method described by Materán *et al.* (2009) with some adaptations: 2 g of frozen tissue were grounded to powder under liquid nitrogen, 5 ml of 80 % Methanol were added and the samples were extracted at 4 °C overnight. Samples were centrifuged at 4000 rpm for 5 min, the supernatant was transferred to a flask and methanol was evaporated. The pH was adjusted to values between 8-9 with a phosphate buffer; 1 ml of ethyl acetate was added and samples were centrifuged at 4000 rpm for 5 min; after discarding the supernatant, the pH was adjusted to 2-3 (with 1N HCl), 2 ml of ethyl acetate were added and the samples were centrifuged at 4000 rpm for 5 min. The supernatant was removed and the ethyl acetate fraction was evaporated. The dry residue was re-suspended in methanol, filtered in brown vials and injected into a 1260 Infinity HPLC-DAD System (Agilent Technologies, Cernusco sul Naviglio, Milano, Italy). ABA was separated on a Purosphere® STAR RP-18, 5 µm, LiChroCART (250-4) (Merck, Darmstadt, Germany) column thermostated at 35 °C. The solvent gradient used was 100 % A (94.9 % H_2O: 5 % CH_3CN: 0.1 % HCOOH) to 100 % B (5 % H_2O: 94.9 % CH_3CN: 0.1 % HCOOH) over 20 min. Solvent B was held at 100 % for 10 min then the solvent returned to 100 % A (Forcat *et al.* 2008). The flow rate into the column was set at 0.5 ml/min. DAD detection was performed at 262 nm, acquiring spectra in the range 190/700 nm.

To quantify ABA concentration in leaf samples the external standard method was used by building a calibration curve with (±)- Abscisic acid, ≥ 98.5 % (Sigma Aldrich SRL, Milan, Italy) concentration ranging from 13.5 to 54.0 mg L^{-1}; ABA identification was performed on the basis of retention times and of DAD spectrum comparison respect to the standard solution.

Statistical analysis

Regression coefficients were obtained using Excel (Microsoft, Redmond, WA, USA), and statistical analysis was performed with univariate analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA) to reveal differences among cultivars and soils, by using IBM SPSS statistics 20.0 software package (SPSS, Chicago, IL). Differences between means were revealed by Tukey test ($p < 0.05$).

Results

Interrelationships between stomatal conductance and soil and stem water potential in different soils and cultivars

Our observations excluded the initial phase of optimal water availability and focused on the dynamics of water relations evolving from mild (day 1 of measurements) to extreme drought, as shown in Fig. 1. The soil water content between WR and WD soils was very different from the beginning, however, the dynamics of the daily averages of Ψ_{stem} and g_s did not express constant differences between soils and cultivars along the period of the trial. The proportion of embolized vessels at petiole level (PLC) was higher on WD soil than on WR for most of the trial, but not constantly along the trial.

In spite of that, the relationship between Ψ_{stem} and θ highlights how the two substrates are distinct for their effect on plant water status (Fig. 2). These differences are already evident at mild water stress conditions (Ψ_{stem} around -0.5 MPa) and while on WR soil the two cultivars show a linear relationship with Ψ_{stem} decreasing with decreasing θ (expressed as small, negative slope of regression lines), on WD the θ is so reduced that

Ψ_{stem} changes substantially for any small variation of θ (expressed as higher, negative slope of regression lines).

The measured Ψ_{stem} was then combined with the calculated soil water potential (Ψ_{soil}) (Fig. 3). The obtained curves show that during water stress Ψ_{stem} declined following a decrease in Ψ_{soil}. In Cabernet Sauvignon this plant adaptation was evident at mild stress conditions, and apparently delayed (and/or less effective) in Syrah.

The response of g_s to Ψ_{stem} was maximum at the beginning of the trial with an overlap of the two curves representing the two cultivars at around -1.4 MPa (Fig. 4a). In comparison to Syrah Cabernet Sauvignon showed lower g_s under mild water stress conditions without strong changes under severe water stress conditions characterising its isohydric behaviour. Our experiment focuses on results obtained under stress, but hypothetical relationships preceding limiting conditions can be drafted: in these conditions Cabernet Sauvignon would probably have shown a steep adaptation to water stress, while Syrah progressively coupled stomatal function with decreasing plant water status (Fig. 4a). When splitting the two curves for the soil plots, further observations can be collected (Fig. 4b). The two cultivars on WD soil maximize their differences, whereas on WR soil they become minimized. Syrah maintains generally higher g_s values than Cabernet Sauvignon, but, while, at a given Ψ_{stem}, in Syrah g_s is higher on WD than on WR soil, the opposite happens in Cabernet Sauvignon.

When these results are presented in form of average values, as illustrated in Fig. 5, all these differences in g_s of the two cultivars appear significantly valid at Ψ_{stem} not lower than -1 MPa, whereas no significant differences between g_s of the different cultivars occur at Ψ_{stem} lower than -1 MPa.

By sorting all measurements of stomatal conductance and stem water potential in three homogenous groups according to decreasing levels of soil water potential, it is possible to run a statistical analysis of results collected at comparable level of soil water availability (Table 1). At highest levels of soil water potential (mild water stress) the cultivar and not the soil significantly drives stomatal conductance, buffering stem water potential adjustments. When water availability in soil further decreases (intermediate water stress) soil properties significantly influence stomatal response. In such conditions, in WR soils a stomatal closure is induced to maintain high levels of stem water potential. In Cabernet Sauvignon the putative isohydric control on water potential is not so effective, as in parallel to a not significant stomatal closure, plants respond to water deprivation with a decrease in water potential. Under severe water stress , however, stomatal control does not avoid decrease on water potential. At these severe levels of water deprivation, soil properties do not influence g_s/Ψ_{stem} response.

Embolism-related and hormone-driven plant adaptations to water stress

While observations concerning g_s are relevant for level of stress not higher than -1MPa, the level of embolism quantified as percent loss of hydraulic conductivity (PLC) provides relevant results also at more extreme conditions (Fig. 6). The differences observed between the two soils are statistically significant ($P < 0.05$) with the vines on WD substrates showing a significantly higher PLC compared to WR substrates at Ψ_{stem} < -1 MPa.

The analysis of the ABA content in leaves showed that the relationship between ABA concentration and g_s was consistently dependent on soil type for Syrah but not for Cabernet Sauvignon (Fig. 7a), variety where stomatal control was tighter (Fig. 7b). In both varieties, significantly in Syrah, the WR soil induces an increase of ABA content in leaf (Fig. 7b).

Discussion

The aim of this study was to investigate how soil water-holding capacity could influence hydraulic and hormone-driven reactions of two cultivars putatively recognised as different in their stomatal response to water stress: Cabernet Sauvignon and Syrah.

Hydraulic control of water stress

Water stress effects were already apparent at mild water stress conditions (Ψ_{stem} around -0.5 MPa), when plants started to experience different shrinking capacities of the two substrates. According to Whitmore and Whalley (2009), in fact, when a shrinking soil dries, as WR substrate of our pots, its degree of saturation is kept small in comparison with a drying rigid soil, such as the WD soil of this experiment (Fig. 1). In WD soils, the matric potential becomes negative much faster, lowering the level of saturation after a much smaller amount of water is removed by roots

In addition to the soil effect, with $\Delta\Psi$ between soil and stem higher for Cabernet Sauvignon than for Syrah, the two cultivars expressed a different capacity of water extraction from the substrate (Fig. 3), requiring to the former a higher energy in order to keep the water flow under increasing stress conditions. Furthermore, and probably related to the above-mentioned reason, Syrah displays higher g_s values than Cabernet Sauvignon, especially during early phases of water stress (mild water stress) (Fig. 4). On the other hand, Cabernet Sauvignon would preserve soil moisture more efficiently than Syrah, imposing at the same time a sensitive control to Ψ_{stem} while Ψ_{soil} decreases (Fig. 3). This result is consistent with putative near-anisohydric behaviour for Syrah and near-isohydric behaviour for Cabernet Sauvignon and with results recently obtained in an experiment by Hochberg *et al.* (2013). Also a lower leaf area of the canopy could preserve soil moisture, but our pot plants have been uniformed to have not different leaf area. The curves obtained from the four combinations soil/cultivar (Fig. 4*b*) could be thus explained by the fact that in water-stress conditions near-anisohydric varieties do not promptly regulate their stomatal conductance and therefore their transpiration rate (which was the case of WD substrate, Fig. 2). On the contrary, near-isohydric varieties, by tightly regulating the stomatal aperture, limit more the waste of water resources. Furthermore, it can be observed how the two curves on WR substrate are closer between each other than to the respective cultivar-correspondent on WD. As already observed under field conditions (Tramontini *et al.* 2013*a*), the expression of plant reactions to water stress seems to be buffered on clay soils. This could be due to the higher capacity of this kind of soils to hold water and release it gradually to the plant. It could be hypothesized that WR substrate produces an effect similar to that of clay soil, submitting the potted roots to transient drought conditions (produced by the daily fluctuations of dehydration during the day and rehydration during the night) able to interfere with the physical and hormonal signalling between roots and stem. However, as illustrated in Fig. 5, all these differences in g_s are significantly valid at Ψ_{stem} not lower than -1 MPa. When water stress becomes more severe, stomatal regulation is hydraulically controlled and a feedback on stomatal function derives from the metabolic plant control. Under increasing water stress, the limitations to photosynthesis pass gradually from a stomatal control to a metabolic control (Flexas *et al.* 2004 and 2006). Due to this, the differences between iso- and anisohydric behaviours are evident between mild and moderate water stress, where the expression of the limitations imposed at stomatal level are maximised. In our results, at these conditions, the average g_s is significantly different between varieties but not between substrates (under each variety), although on WD the differences remain evident. Concerning the consequent risk of cavitation, Syrah on both soils and Cabernet Sauvignon on WD have an increase in embolism formation, expressed in terms of xylem conductivity losses, of 32–36%, moving from $\Psi_{stem} > -1$ MPa to $\Psi_{stem} < -1$ MPa. Only Cabernet Sauvignon on WR soil shows higher embolism formation at $\Psi_{stem} > -1$ MPa than at $\Psi_{stem} < -1$ MPa. An explanation of this phenomenon would require the support of further data concerning, for example, the implication of the chemical signalling (in particular ABA) in the transpiration control. Soar *et al.* (2006) have in fact demonstrated the contribution of ABA to the differential response of g_s in iso- and anisohydric cultivars.

Abscisic-acid control on stomatal conductance

On the near-isohydric cultivar, Cabernet Sauvignon, expressing very similar level of cavitation on the two soils at $\Psi_{stem} > -1$ MPa, we could observe a more stable ABA signal, independently from the soil (Fig. 7), similarly to observations by Puértolas *et al.* (2013) using *Phaseolus vulgaris* L. In contrast, in Syrah, showing two levels of cavitation on the two soils both at moderate and at higher stress level, also the curves of ABA concentration in leaves were clearly distinguished, between the leaves of plants on WR soil richer on the hormone than those on WD soil, showing a substrate-dependant ABA concentration, as observed by Dodd *et al.* (2010) on *Helianthus annuus* L. In order to analyze better this result we suggest comparing it with that on Fig. 4b: contrary to initial expectations, Syrah has generally higher g_s on WD than on WR soil, and this may be due to the specific circumstances produced by the WR soil, as above-mentioned, favouring the release of the hormone (ABA) in the leaf. As recently observed by Brodribb and McAdam (2013) on two conifer species, the isohydric stomatal regulation can be identified as an ABA-driven stomatal closure, while the anisohydric is at least initially water potential-driven. The same appears to be true on our two grapevine cultivars: ABA control on g_s is tight in Cabernet Sauvignon and it is independent to soil properties. In Syrah plants potted on WD soil a similar ABA control on stomatal conductance subsists. However, when the anisohydric Syrah grows onto the WR soil, an additional ABA leaf biosynthesis or accumulation is recordable. The WR-induced raise in ABA allows stomatal control limiting the anisohydric response, as it happens when anisohydric grapevines are deficit-irrigated upon partial root zone drying (Stoll *et al.* 2000; Romero *et al.* 2012).

Hints for future research and speculations

Our results are in line with those recently presented by Hochberg *et al.* (2013) on a similar work done on the same two varieties and with the general consideration on the differential photoprotective response to stress in iso- and anisohydric cultivars (Pou *et al.* 2012). We would expect that plant productivity of Cabernet Sauvignon, due to the ABA-driven stomatal closure and its putatively stronger downregulation of photosynthesis, is less influenced by the soil characteristics than Syrah.

The results of our current study combined with the ecological and oenological characteristics of the two genotypes, seem to find coherence: Cabernet Sauvignon, the more isohydric variety, thanks to a tight stomatal control, conserves varietal characteristics on the grape independently from the growing conditions. From a viticultural point of view, the avoidance of extreme conditions (and of the consequent recovery phases) to which Syrah is more prone, allows this variety to buffer vintage differences . Hence, the more anisohydric variety, seems to base its stomatal control more on hydraulic signals. This could be hypothesized as the effect of a higher involvement of long term adaptation mechanisms, such as anatomic modifications, and the development of a product which strongly varies according to the characteristics of the substrate. Both are expressions of the *terroir* concept favouring different components and mechanisms to adapt.

Although our results have been obtained on potted plants, where the nature of the substrate and the available volume for root development are a limiting projection of the edaphic condition of a vineyard, nevertheless they could be of support in the interpretation of *terroir* expression previously introduced by the same authors (Tramontini *et al.* 2013*a*). The isohydric Cabernet Sauvignon can adapt to a variety of climates and soils and, in spite of that, maintain certain organoleptic traits in the final product. It is considered extremely capable to express the characteristics of a given *terroir* and, due to that, has been for a long time the world's most widely planted premium red wine grape (Robinson 2006). The anisohydric Syrah, on the other hand, is a very common commercial variety (the world's 7th most grown grape in 2004, still according to Robinson 2006) particularly distributed in warmer regions, from which very diverse wines can be produced.

Furthermore, ABA plays a key role by stimulating the activation of the anthocyanin and flavonoids biosynthesis pathway (Davies and Böttcher 2009; Ferrandino and Lovisolo 2014). Both, its impact on water relations and on berry metabolism may contribute to a differential berry quality.This hypothesis could represent a relevant topic for further studies in field conditions, where also long terms mechanisms of adaptation and more complex dynamics of hormonal signalling (Dodd 2013) can be observed, and extended to other varieties, considering the main mechanisms involved in the *terroir* expression.

Conclusions

In conclusion, we reported a hydraulic control of stomatal responses at the base of the near-anisohydric Syrah adaptations to water stress, in contrast to an ABA-induced stomatal control in the near-isohydric Cabernet Sauvignon. Also is Syrah, however, the hormone-related response could be effective when soil properties allowed for higher water storage buffering hydraulic adaptations.

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Figure legends

Figure 1. (a) Dynamics of soil moisture (θ, %), (b) stem water potential (Ψ_{stem}, MPa), (c) stomatal conductance (g_s, mmol m^{-2} s^{-1}), and percent loss of (d) conductivity due to embolisms (PLC, %), during the days of the trial. Measurements were conducted on plants of Cabernet Sauvignon (*circles*) and Syrah (*triangles*) on water draining (WD, *white*) and water retaining (WR, *black*) soils. Means ± std err. *Diamonds* in frame (d) represent the mean value of the day for both cultivars grouped.

Figure 2. Relationship between stem water potential (Ψ_{stem}, MPa) and soil moisture (θ, %) measured on plants of Cabernet Sauvignon (*circles*) and Syrah (*triangles*) on water draining (WD, *white*) and water retaining (WR, *black*) soils. Arrows on the x axis point to maximum water-holding capacity of the two soils (% water at -0.01 MPa).

Figure 3. Relationship between stem water potential (Ψ_{stem}, MPa) and soil water potential (Ψ_{soil}, MPa) measured on plants of Cabernet Sauvignon (*circles*) and Syrah (*triangles*) on water draining (WD, *white*) and water retaining (WR, *black*) soils. Ψ_{stem} was obtained from direct measures while Ψ_{soil} from the derived equations of Ψ_{soil} and θ.

Figure 4. Interrelationship between stomatal conductance (g_s, mmol m^{-2} s^{-1}) and stem water potential (Ψ_{stem}, MPa) measured on plants of Cabernet Sauvignon (*circles*) and Syrah (*triangles*) on water draining (WD, *white*) and water retaining (WR, *black*) soils. The two figures present the same data clustered only for varieties (a) and for the varieties on each soil (b). In addition, in Fig. 4a, an arbitrary hypothetical curve preceding water stress has been identified with a dashed line.

Figure 5. Average values of leaf stomatal conductance (g_s, mmol m^{-2} s^{-1}) measured on plants of Cabernet Sauvignon on water retaining soil (WR, *black*) and on water draining soil (WD, *light grey*) and on Syrah plants on WR (*dark grey*) and on WD (*white*). Data have been clustered for those collected between mild and moderate water stress (Ψ_{stem} > -1 MPa) and high water stress (Ψ_{stem} < -1 MPa). Values of bars topped by common letters are not significantly different, while different letters identify significantly different groups ($P<0.05$ (*), $P<0.01$ (**); Tukey Test).

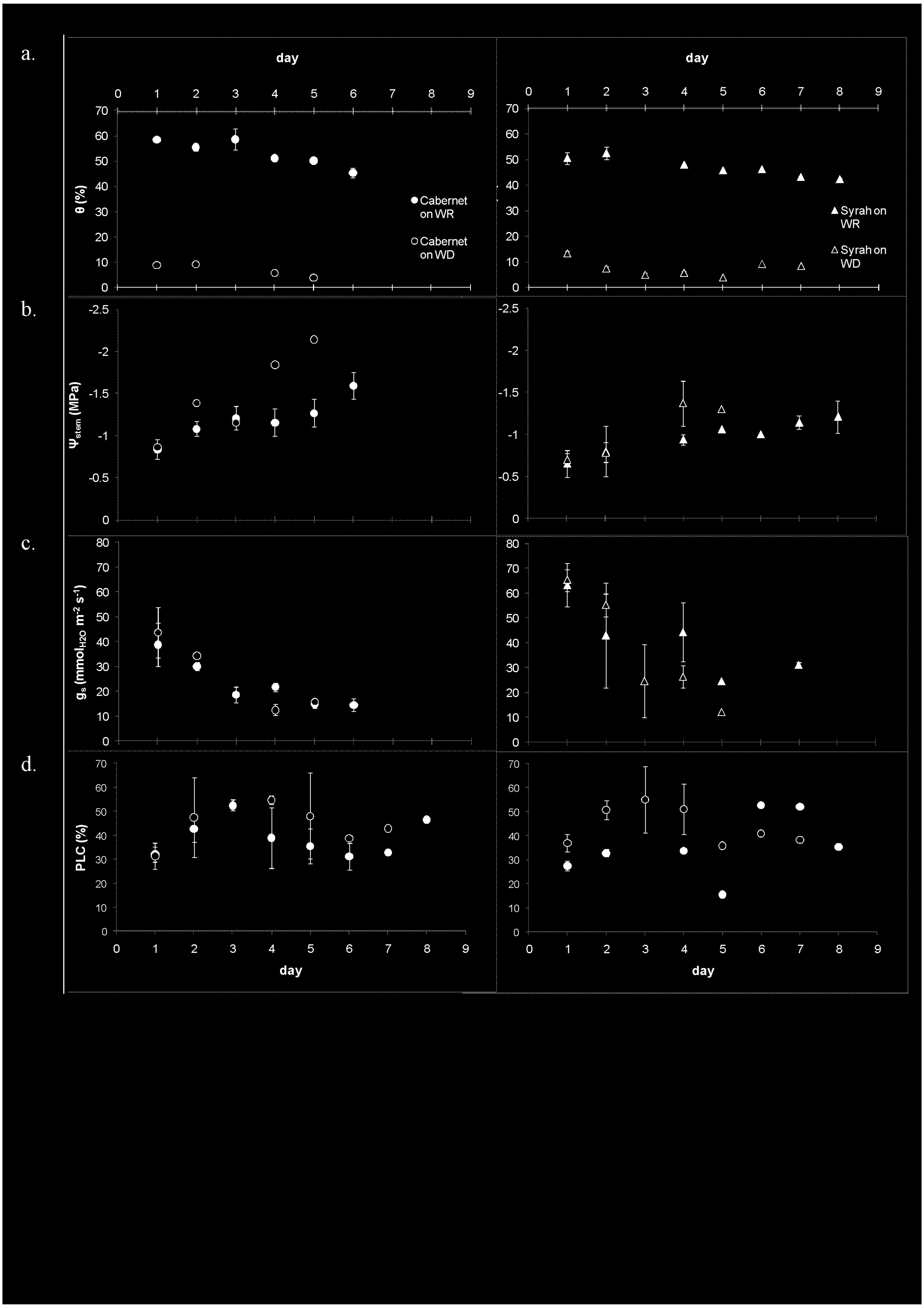
Figure 6. Average values of percent loss of conductivity (PLC, %) due to embolism formation, measured on leaf petioles of Cabernet Sauvignon on water retaining soil (WR, *black*) and on water draining soil (WD, *light grey*) and on Syrah plants on WR (*dark grey*) and on WD (*white*). Data have been clustered for those collected between mild and moderate water stress (Ψ_{stem} > -1 MPa) and high water stress (Ψ_{stem} < -1 MPa). Values of bars topped by common letters are not significantly different, while different letters identify significantly different groups (P<0.05 (*), P<0.01 (**); Tukey Test).

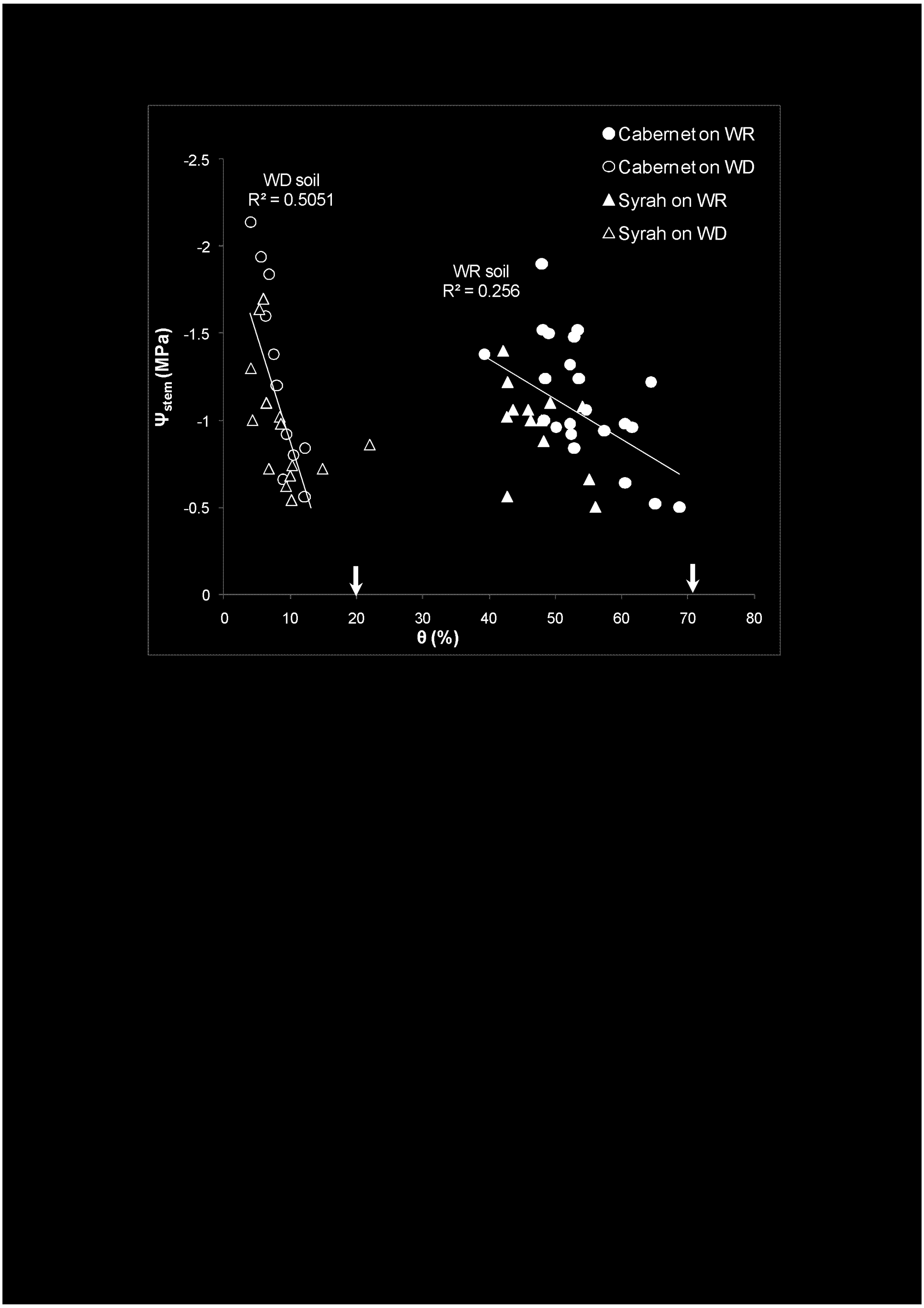
Figure 7 a and b. Relationship between stomatal conductance (g_s, mmol m^{-2} s^{-1}) and abscisic acid (ABA) concentration (ng g^{-1} fw) in leaf samples on plants of Cabernet Sauvignon (*circles*) and Syrah (*triangles*) on water draining (WD, *white*) and water retaining (WR, *black*) soils. In frame (a), continuous lines represent the two curves obtained for Cabernet Sauvignon and dashed lines for Syrah. In frame (b), means ± std errors are displayed.

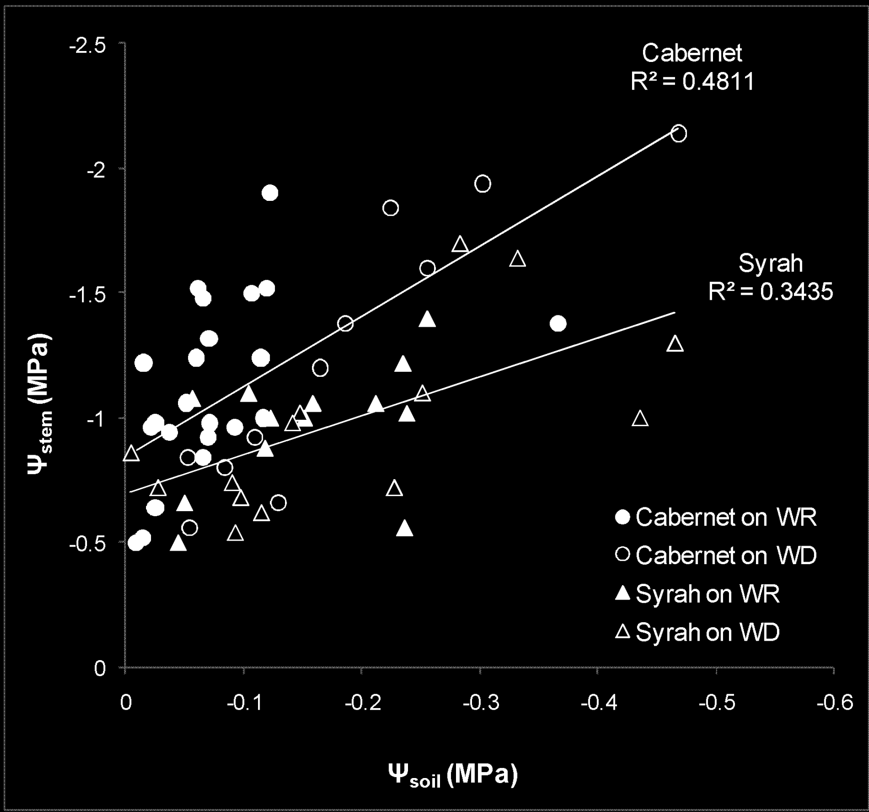
Water stress		Ψ_{stem}		g_s	
Mild (Ψ_{soil} >-0.083)	**Cabernet Sauvignon**	-0.972	n.s.	36.1	b
	Syrah	-0.764	n.s.	75.2	a
Intermediate (-0.083 > Ψ_{soil} > -0.212)	**Cabernet Sauvignon**	-1.189	b	33.4	n.s.
	Syrah	-0.875	a	55.3	n.s.
Severe (Ψ_{soil} <-0.212)	**Cabernet Sauvignon**	-1.780	b	14.7	b
	Syrah	-1.087	a	35.2	a
Mild (Ψ_{soil} >-0.083)	**water retaining soil (WR)**	-0.964	n.s.	41.9	n.s.
	water draining soil (WD)	-0.745	n.s.	60.9	n.s.
Intermediate (-0.083 > Ψ_{soil} > -0.212)	**water retaining soil (WR)**	-1.196	n.s	27.9	b
	water draining soil (WD)	-0.867	n.s	60.8	a
Severe	**water retaining soil (WR)**	-0.994	n.s.	19.5	n.s.

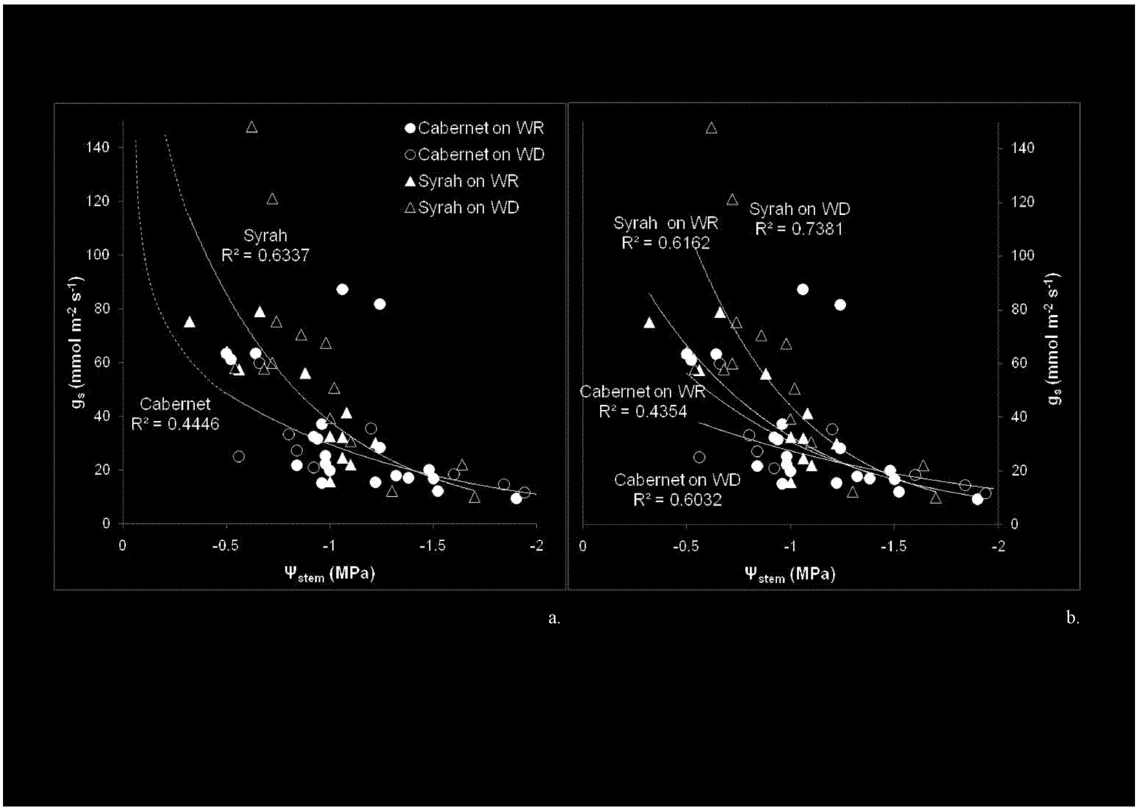
(Ψ_{soil} <-0.212)	**water draining soil (WD)**	-1.498	n.s.	22.3	n.s.

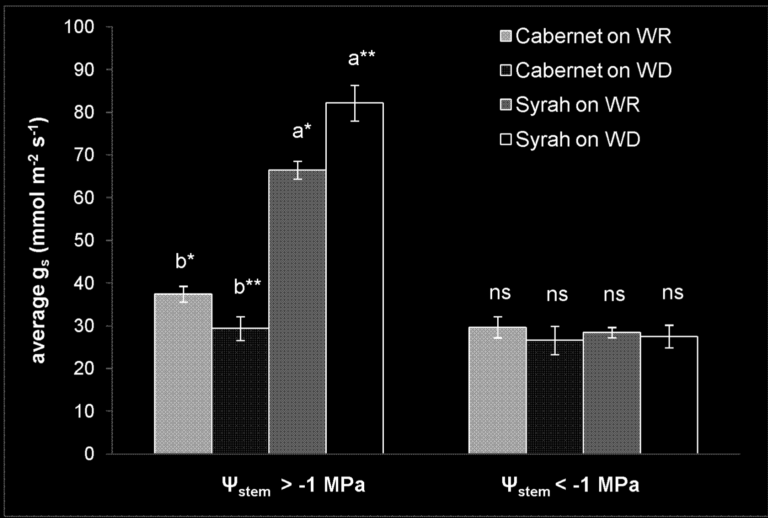
Table 1: influence of cultivar and soil water-holding capacity on stem water potential (Ψ_{stem}) and stomatal conductance (g_s). Data were divided in three classes of soil water potential (Ψ_{soil}) values: mild (Ψ_{soil} >-0.083), intermediate (-0.083 > Ψ_{soil} > -0.212) and severe water stress (Ψ_{soil} <-0.212), and processed separately for the two effects of cultivar and soil. Different letters indicate significant differences among means, *F*-test, $P<0.05$, post hoc Tukey's test.

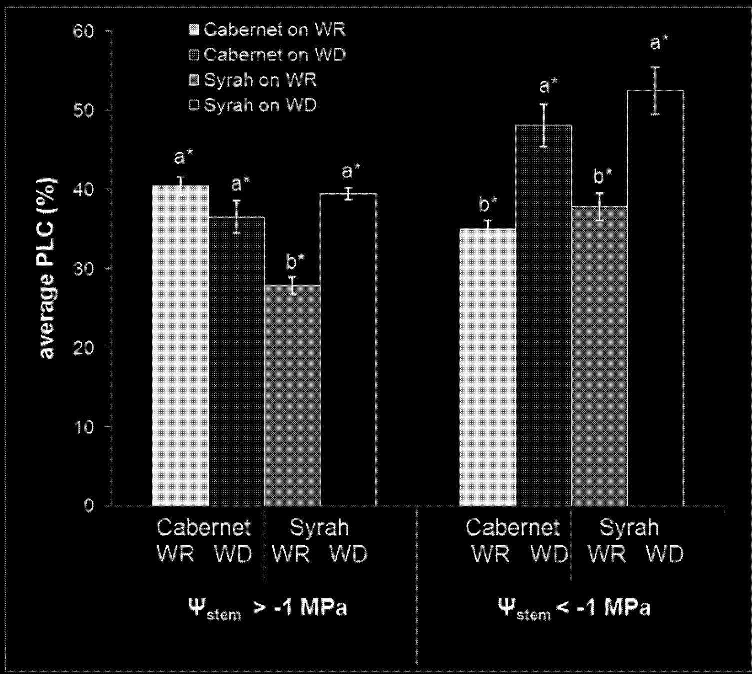

a.
b.
c.
d.
day
θ (%)
Cabernet on WR
Cabernet on WD
Syrah on WR
Syrah on WD
Ψstem (MPa)
gs (mmolH2O m-2 s-1)
PLC (%)
day

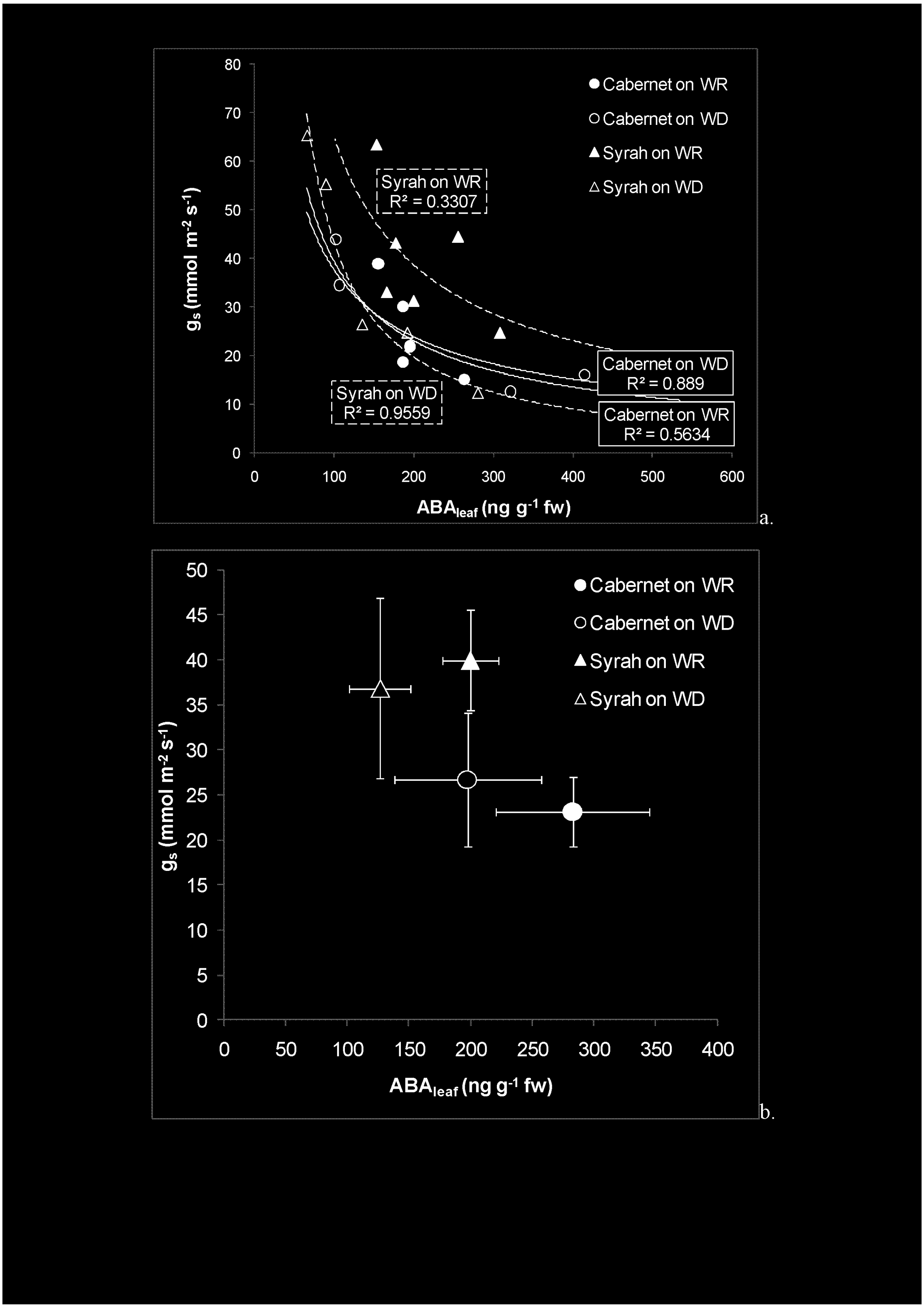

Cabernet on WR
Cabernet on WD
Syrah on WR
Syrah on WD
WD soil
R² = 0.5051
WR soil
R² = 0.256
Ψstem (MPa)
θ (%)
-2.5
-2
-1.5
-1
-0.5
0
0
10
20
30
40
50
60
70
80


Cabernet
R² = 0.4811
Syrah
R² = 0.3435
Cabernet on WR
Cabernet on WD
Syrah on WR
Syrah on WD
Ψstem (MPa)
Ψsoil (MPa)
-2.5
-2
-1.5
-1
-0.5
0
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6


Cabernet on WR
Cabernet on WD
Syrah on WR
Syrah on WD
Syrah
R² = 0.6337
Cabernet
R² = 0.4446
Syrah on WR
R² = 0.6162
Syrah on WD
R² = 0.7381
Cabernet on WR
R² = 0.4354
Cabernet on WD
R² = 0.6032
g_s (mmol m^{-2} s^{-1})
Ψ_{stem} (MPa)
0
20
40
60
80
100
120
140
0
-0.5
-1
-1.5
-2
a.
b.


average g_s (mmol m^{-2} s^{-1})
100
90
80
70
60
50
40
30
20
10
0
Cabernet on WR
Cabernet on WD
Syrah on WR
Syrah on WD
b*
b**
a*
a**
ns
ns
ns
ns
Ψ_{stem} > -1 MPa
Ψ_{stem} < -1 MPa


Cabernet on WR
Cabernet on WD
Syrah on WR
Syrah on WD
average PLC (%)
60
50
40
30
20
10
0
a*
a*
b*
a*
b*
a*
b*
a*
Cabernet
WR WD
Syrah
WR WD
Cabernet
WR WD
Syrah
WR WD
Ψstem > -1 MPa
Ψstem < -1 MPa


80
70
60
50
40
30
20
10
0
0
100
200
300
400
500
600
gs (mmol m-2 s-1)
ABAleaf (ng g-1 fw)
Cabernet on WR
Cabernet on WD
Syrah on WR
Syrah on WD
Syrah on WR
R² = 0.3307
Syrah on WD
R² = 0.9559
Cabernet on WD
R² = 0.889
Cabernet on WR
R² = 0.5634
a.
50
45
40
35
30
25
20
15
10
5
0
0
50
100
150
200
250
300
350
400
gs (mmol m-2 s-1)
ABAleaf (ng g-1 fw)
Cabernet on WR
Cabernet on WD
Syrah on WR
Syrah on WD
b.