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

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1 Soil water-holding capacity mediates hydraulic and hormonal signals of 2 near-isohydric and near-anisohydric *Vitis* cultivars in potted grapevines.

- 3 **Abridged title:** Soil and genotype influence on grapevine response to drought.
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14 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.

22 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. 29 Xylem embolism contributed to plant adaptation to soil water deprivation: in both 30 cultivars during late phases of water stress, however, in Syrah, already at moderate early 31 stress levels. By contrast, Syrah showed a less effective stomatal control of drought than 32 Cabernet Sauvignon. The abscisic acid (ABA) influenced tightly the stomatal 33 conductance of Cabernet Sauvignon on both pot soils. In the near-anisohydric variety 34 Syrah an ABA-related stomatal closure was induced in WR soil to maintain high levels 35 of water potential, showing that a soil-related hormonal root-to-shoot signal causing 36 37 stomatal closure superimposes on the putatively variety-induced anisohydric response to water stress. 38

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

41 Introduction

42 Grapevine (*Vitis vinifera* L.) is a species expressing both isohydric and anisohydric

43 behaviours, not only depending from genotype (Schultz 2003), but also from the

44 influence of growing conditions or seasonality (Chaves *et al.* 2010, de Souza *et al.*

45 2003) or from the environmental conditions to which the plant was exposed (Collins *et*

46 *al.* 2010; Lovisolo *et al.* 2010; Pou *et al.* 2012; Tramontini *et al.* 2013*a*).

47 Although the genotype itself is not sufficient to preview the physiological behaviour of grapevine plants, some cultivars have been more frequently observed expressing 48 49 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 50 conditions (Schultz 2003; Rogiers et al. 2009; Soar et al. 2009) or in pots (Soar et al. 51 2006). Cabernet Sauvignon, on the other hand, has been more frequently observed to 52 display a response to water deprivation nearer to isohydric type (Hochberg et al. 2013). 53 Owing to the differential response observed on these two cultivars under the same water 54 55 conditions, Cabernet Sauvignon and Syrah have already been coupled in comparative experiments (Chalmers 2007; Petrie and Sadras 2008; Rogiers et al. 2009; Hochberg et 56 57 al. 2013) and can therefore be selected as efficient models for representing iso- and anisohydric behaviours. 58

The stomatal control, which is an endogenous, but highly variable character, was 59 considered in combination with the soil effect. Soil is in fact another crucial component 60 in grape and wine production, not only because it determines the water and nutrients 61 availability for the plant and therefore its productive performances, but also for its 62 specific implication in the "terroir effect" in viticulture (Bodin and Morlat 2006; van 63 Leeuwen et al. 2009). In spite of the acknowledged importance on grape and wine 64 production, not many studies attempted to quantify its effects with comparative trials. 65 For this reason, in the presented work, we decided to focus the attention only on the 66 differences produced by two soils in terms of soil texture and related water availability 67 provided to the plant: one single aspect which is, however, strongly influenced by 68 69 physical, chemical, and biological properties of the substrate. When a soil dries, in fact, 70 the increasing drought affects the plant in multiple and complex ways (Whitmore and 71 Whalley 2009).

Cavitation of the xylem vessels is a very relevant consequence of the limited soil 72 moisture, as it can produce dramatic consequences by reducing the hydraulic 73 conductivity of the vascular tissues and impairing the possibility for the plant to replace 74 transpired water (Brodersen et al. 2013). It is also one of the most studied effects of 75 drought in grapevine, in combination with loss in hydraulic conductance (Lovisolo and 76 Tramontini 2010). In leaves, cavitation and consequent embolism formation affect 77 78 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.* 79 2011). On the other hand, the entity of damage produced by cavitation and the break 80 81 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 82 83 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).

96 Furthermore, in grapevine metabolic and hydraulic behaviour have shown to be related,

97 according to the observations recently published by Hochberg *et al.* (2013) from a study

98 conducted on Cabernet Sauvignon and Syrah plants too. In this work the more

99 anisohydric grapevine cultivar showed higher water uptake and higher g_s than the near-100 isohydric cultivar.

101 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

103 ecophysiological behaviour, from the point of view of the hydraulic balance of the plant

104 (i.e. water potential, stomatal control, embolism formation), and its hormonal(ABA)

105 control of water losses.

106 Materials and Methods

107 *Plant material and growing conditions*

The trial was conducted in August 2012 at Hochschule Geisenheim University 108 109 (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 110 hybrids of Vitis berlandieri × Vitis riparia ('161-49 Couderc'for 'Cabernet Sauvignon' 111 and '420A Millardet Et De Grasset' for 'Syrah') of comparable characteristics (Whiting 112 2004), especially in controlling the interrelationship between leaf or stem water 113 potential and stomatal conductance (Tramontini et al. 2013b). The plants were 114 115 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, 116 117 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 118 Classic, Einheitserde-Einheitserde- und Humuswerke Gebr. Patzer GmbH & Co.KG, 119 Sinntal, Germany; consisting of 55% white peat, 30% clay, 15% sod peat; chemical 120

properties pH (CaCl₂) 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.

124 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 125 126 nitrogen availability. Soil nitrogen content after the fertilization was estimated according to Robinson recommendations (1988), confirming that at the beginning of the 127 128 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} 129 \leq -0.5 MPa), such as four days after interruption of irrigation. In that moment plants had 130 14.4 ± 2.8 leaves with no significant differences between cultivars or soils. Each plant 131 132 was excluded from the trial when wilting was observed.

- 133 Soil water content (θ , %), soil water potential (Ψ_{soil} , MPa), stem water potential (Ψ_{stem} ,
- 134 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
- 137 circadian expression in cell water channels (Uehlein and Kaldenhoff 2006).

138 Water relations

- 139 Soil water content (θ) was gravimetrically determined by collecting daily approximately
- 140 10 ml of soil from three different points and depths in each pot (5, 10, 15 cm depth at
- 141 the half of rays 120° distant one from the other). The soil was weighed, oven-dried at
- 142 100 °C for 24 h and then re-weighed to assess water content. At the same time, the
- 143 water retention curves for the two soils were assessed with pressure plate measurements
- 144 of the potting substrate (Richards 1965), obtaining two equations:
- 145 WR soil $-\Psi_{soil} = 53.791 * e^{-0.127* \theta}$
- 146 WD soil $-\Psi_{soil} = 1.3423 * e^{-0.264 * \theta}$
- 147 The obtained relationships allowed for the calculation of Ψ_{soil} based on θ .

148 Ψ_{stem} was measured on mature, undamaged and non-senescent leaves using a pressure

149 chamber (Soilmoisture Corp., Santa Barbara, CA, USA) (Scholander *et al.* 1965) at

150 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

152 measurements were taken.

153 *Xylem embolism*

Daily determination of xylem embolisms in leaf petioles, induced by the presence of air 154 bubbles in xylem vessels, was carried out around midday using a high-pressure 155 flowmeter (HPFM, Dynamax Inc., Houston, TX, USA) (Tyree et al. 1995). As the 156 assessment of embolism extent is a destructive analysis, leaf petioles were used as a 157 proxy of the plant behaviour (Lovisolo et al. 2008; Perrone et al. 2012). During the 158 whole duration of the experiment macro- and microbubbles were regularly flushed out 159 of the system according to the manufacturer's instruction manual and the mismatch 160 between the two pressure transducers was controlled daily by running the 'Set Zero' 161 162 routine before measuring.

For each determination of percent loss of conductivity (PLC), the petioles and leaves 163 164 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 165 above the petiole insertion a few seconds after starting the measurement. The initial 166 hydraulic conductance K_{hi} was determined applying an initial pressure of ~20 kPa for 3 167 min. Distilled and degassed water with an addition of 10 mmol L^{-1} KCl was used as 168 perfusion liquid. Petioles were then flushed for 3 min applying a transient increase of 169 pressure until a pressure of ~550 kPa was reached. This pressure was kept constant for 3 170 min. To determine the final hydraulic conductance K_{hf} the pressure was downregulated 171 to ~ 20 kPa and held constant for 3 min. To calculate K_{hi} and K_{hf} average values of the 172 hydraulic conductance of the respective timespans were used. 173

174 Data were displayed and stored using the software HPFM95-XP Version 1.12

175 (Dynamax Inc.) and exported and processed using Microsoft Excel.

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

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

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

180 Stomatal conductance

181 Measurements of g_s were carried out on adult, non-senescent leaves that were well-

182 exposed to direct sunlight. G_s was measured using a porometer (AP4, Delta-T Devices

183 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.

185 Analysis of abscisic acid (ABA) in leaves

186 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 187 were grounded to powder under liquid nitrogen, 5 ml of 80 % Methanol were added and 188 the samples were extracted at 4 °C overnight. Samples were centrifuged at 4000 rpm for 189 5 min, the supernatant was transferred to a flask and methanol was evaporated. The pH 190 was adjusted to values between 8-9 with a phosphate buffer; 1 ml of ethyl acetate was 191 added and samples were centrifuged at 4000 rpm for 5 min; after discarding the 192 supernatant, the pH was adjusted to 2-3 (with 1N HCl), 2 ml of ethyl acetate were added 193 and the samples were centrifuged at 4000 rpm for 5 min. The supernatant was removed 194 195 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 196 197 (Agilent Technologies, Cernusco sul Naviglio, Milano, Italy). ABA was separated on a Purosphere® STAR RP-18, 5 µm, LiChroCART (250-4) (Merck, Darmstadt, Germany) 198 column thermostated at 35 °C. The solvent gradient used was 100 % A (94.9 % H₂O: 5 199 % CH₃CN: 0.1 % HCOOH) to 100 % B (5 % H₂O: 94.9 % CH₃CN: 0.1 % HCOOH) 200 over 20 min. Solvent B was held at 100 % for 10 min then the solvent returned to 100 % 201 A (Forcat et al. 2008). The flow rate into the column was set at 0.5 ml/min. DAD 202 203 detection was performed at 262 nm, acquiring spectra in the range 190/700 nm.

204 To quantify ABA concentration in leaf samples the external standard method was used

by building a calibration curve with (\pm)- Abscisic acid, \geq 98.5 % (Sigma Aldrich SRL,

Milan, Italy) concentration ranging from 13.5 to 54.0 mg L^{-1} ; ABA identification was

- 207 performed on the basis of retention times and of DAD spectrum comparison respect to
- the standard solution.

209 *Statistical analysis*

- 210 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).
- 214 Differences between means were revealed by Tukey test (p < 0.05).

215

216 **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
- 230 (expressed as small, negative slope of regression lines), on WD the θ is so reduced that

231 Ψ_{stem} changes substantially for any small variation of θ (expressed as higher, negative 232 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 237 of the two curves representing the two cultivars at around -1.4 MPa (Fig. 4a). In 238 comparison to Syrah Cabernet Sauvignon showed lower g_s under mild water stress 239 conditions without strong changes under severe water stress conditions characterising 240 its isohydric behaviour. Our experiment focuses on results obtained under stress, but 241 hypothetical relationships preceding limiting conditions can be drafted: in these 242 conditions Cabernet Sauvignon would probably have shown a steep adaptation to water 243 stress, while Syrah progressively coupled stomatal function with decreasing plant water 244 245 status (Fig. 4a). When splitting the two curves for the soil plots, further observations can 246 be collected (Fig. 4b). The two cultivars on WD soil maximize their differences, whereas on WR soil they become minimized. Syrah maintains generally higher gs 247 248 values than Cabernet Sauvignon, but, while, at a given Ψ_{stem} , in Syrah gs is higher on

249 WD than on WR soil, the opposite happens in Cabernet Sauvignon.

250 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

253 occur at Ψ_{stem} lower than -1 MPa.

254 By sorting all measurements of stomatal conductance and stem water potential in three

255 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

258 cultivar and not the soil significantly drives stomatal conductance, buffering stem water

259 potential adjustments. When water availability in soil further decreases (intermediate

260 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,
- 265 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.

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

- 268 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)
- 270 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}
- 273 <-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
- 276 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 contentin leaf (Fig. 7b).
- 279 **Discussion**
- 280 The aim of this study was to investigate how soil water-holding capacity could
- 281 influence hydraulic and hormone-driven reactions of two cultivars putatively recognised
- as different in their stomatal response to water stress: Cabernet Sauvignon and Syrah.
- 283 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 aftera 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 291 292 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 293 294 keep the water flow under increasing stress conditions. Furthermore, and probably 295 related to the above-mentioned reason, Syrah displays higher g_s values than Cabernet 296 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 297 298 than Syrah, imposing at the same time a sensitive control to Ψ_{stem} while Ψ_{soil} decreases 299 (Fig. 3). This result is consistent with putative near-anisohydric behaviour for Syrah and 300 near-isohydric behaviour for Cabernet Sauvignon and with results recently obtained in 301 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 302 303 area. The curves obtained from the four combinations soil/cultivar (Fig. 4b) could be thus explained by the fact that in water-stress conditions near-anisohydric varieties do 304 not promptly regulate their stomatal conductance and therefore their transpiration rate 305 (which was the case of WD substrate, Fig. 2). On the contrary, near-isohydric varieties, 306 307 by tightly regulating the stomatal aperture, limit more the waste of water resources. 308 Furthermore, it can be observed how the two curves on WR substrate are closer between 309 each other than to the respective cultivar-correspondent on WD. As already observed 310 under field conditions (Tramontini et al. 2013a), the expression of plant reactions to 311 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 312 313 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 314 315 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, 316 317 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 318 319 hydraulically controlled and a feedback on stomatal function derives from the metabolic 320 plant control. Under increasing water stress, the limitations to photosynthesis pass

321 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 322 between mild and moderate water stress, where the expression of the limitations 323 imposed at stomatal level are maximised. In our results, at these conditions, the average 324 g_s is significantly different between varieties but not between substrates (under each 325 variety), although on WD the differences remain evident. Concerning the consequent 326 327 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%, 328 moving from $\Psi_{stem} > -1$ MPa to $\Psi_{stem} < -1$ MPa. Only Cabernet Sauvignon on WR soil 329 shows higher embolism formation at $\Psi_{\text{stem}} > -1$ MPa than at $\Psi_{\text{stem}} < -1$ MPa. An 330 explanation of this phenomenon would require the support of further data concerning, 331 332 for example, the implication of the chemical signalling (in particular ABA) in the 333 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. 334

335 Abscisic-acid control on stomatal conductance

336 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 337 signal, independently from the soil (Fig. 7), similarly to observations by Puértolas et al. 338 (2013) using Phaseolus vulgaris L. In contrast, in Syrah, showing two levels of 339 340 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 341 342 WR soil richer on the hormone than those on WD soil, showing a substrate-dependent ABA concentration, as observed by Dodd et al. (2010) on Helianthus annuus L. In 343 344 order to analyze better this result we suggest comparing it with that on Fig. 4b: contrary to initial expectations, Syrah has generally higher gs on WD than on WR soil, and this 345 may be due to the specific circumstances produced by the WR soil, as above-mentioned, 346 favouring the release of the hormone (ABA) in the leaf. As recently observed by 347 Brodribb and McAdam (2013) on two conifer species, the isohydric stomatal regulation 348 can be identified as an ABA-driven stomatal closure, while the anisohydric is at least 349 350 initially water potential-driven. The same appears to be true on our two grapevine 351 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 352

353 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.*

357 2000; Romero *et al.* 2012).

358 *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

364 photosynthesis, is less influenced by the soil characteristics than Syrah.

365 The results of our current study combined with the ecological and oenological characteristics of the two genotypes, seem to find coherence: Cabernet Sauvignon, the 366 367 more isohydric variety, thanks to a tight stomatal control, conserves varietal characteristics on the grape independently from the growing conditions. From a 368 369 viticultural point of view, the avoidance of extreme conditions (and of the consequent 370 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 371 more on hydraulic signals. This could be hypothesized as the effect of a higher 372 involvement of long term adaptation mechanisms, such as anatomic modifications, and 373 the development of a product which strongly varies according to the characteristics of 374 375 the substrate. Both are expressions of the terroir concept favouring different components and mechanisms to adapt. 376

377 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

380 interpretation of *terroir* expression previously introduced by the same authors

381 (Tramontini *et al.* 2013*a*). The isohydric Cabernet Sauvignon can adapt to a variety of

382 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.

396 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.

402

403

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554 Figure legends

Figure 1. (a) Dynamics of soil moisture (θ , %), (b) stem water potential (Ψ_{stem} , MPa),

556 (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,

559 *white*) and water retaining (WR, *black*) soils. Means \pm 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 ,

562 %) 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

567 (*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

570 water potential (Ψ_{stem} , MPa) measured on plants of Cabernet Sauvignon (*circles*) and

571 Syrah (*triangles*) on water draining (WD, *white*) and water retaining (WR, *black*) soils.

572 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

574 preceding water stress has been identified with a dashed line.

Figure 5. Average values of leaf stomatal conductance $(g_s, \text{ mmol } m^{-2} s^{-1})$ 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 ($\Psi_{stem} >$

579 -1 MPa) and high water stress ($\Psi_{stem} < -1$ MPa). Values of bars topped by common

580 letters are not significantly different, while different letters identify significantly

581 different groups (*P*<0.05 (*), *P*<0.01 (**); Tukey Test).

- formation, measured on leaf petioles of Cabernet Sauvignon on water retaining soil
- 584 (WR, *black*) and on water draining soil (WD, *light grey*) and on Syrah plants on WR
- 585 (*dark grey*) and on WD (*white*). Data have been clustered for those collected between
- mild and moderate water stress ($\Psi_{stem} > -1$ MPa) and high water stress ($\Psi_{stem} < -1$ MPa).
- 587 Values of bars topped by common letters are not significantly different, while different
- 1588 letters identify significantly different groups (*P*<0.05 (*), *P*<0.01 (**); Tukey Test).
- 589 Figure 7 a and b. Relationship between stomatal conductance $(g_s, \text{ mmol } m^{-2} s^{-1})$ and
- abscisic acid (ABA) concentration (ng g^{-1} fw) in leaf samples on plants of Cabernet
- 591 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 \pm std
- 595

errors are displayed.

Water stress		Ψ _{stem}		gs	
Mild	Cabernet Sauvignon	-0.972	n.s.	36.1	b
$(\Psi_{\text{soil}} > -0.083)$	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	а
Mild $(\Psi_{soil} > -0.083)$	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.

$(\Psi_{soil} <-0.212) \qquad \text{water draining soil (WD)}$	-1.498	n.s.	22.3	n.s.
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597 Table 1: influence of cultivar and soil water-holding capacity on stem water potential

598 (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 ($\Psi_{soil} \le 0.212$), and processed separately for the two effects of

601 cultivar and soil. Different letters indicate significant differences among means, *F*-test,

602 P < 0.05, post hoc Tukey's test.













