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

(Article begins on next page)

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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.**

15 The ecophysiological behaviour of grapevine cultivars in response to drought is

16 influenced by the soil conditions and by the plant genotype. These two components

17 interact through a complex of hydraulic and hormonal signal exchanges occurring

18 between roots and leaves. Our work highlighs the differences in these signals observed

19 in a near-isohydric and a near-anisohydric grapevine cultivars on two soil substrates

20 with different textures, causing different dynamics of water deprivation during an

21 imposed increasing water stress.

22 **Abstract**

23 Grapevine (*Vitis vinifera* L.) expresses different responses to water stress, not only

24 depending from genotype, but also from the influence of vineyard growing conditions

25 or seasonality. We aimed to analyze the effects on drought response of two grapevine

26 cultivars growing on two soils, one water draining (WD) containing sand 80% vol. and

27 the other water retaining (WR), with no sand. Under these two different water-holding

28 capacities Syrah, displaying a near-anisohydric response to water stress, and Cabernet

29 Sauvignon (on the contrary, near-isohydric) were submitted to water stress in a pot trial.

30 Xylem embolism contributed to plant adaptation to soil water deprivation: in both

31 cultivars during late phases of water stress, however, in Syrah, already at moderate early

32 stress levels. By contrast, Syrah showed a less effective stomatal control of drought than

33 Cabernet Sauvignon. The abscisic acid (ABA) influenced tightly the stomatal

34 conductance of Cabernet Sauvignon on both pot soils. In the near-anisohydric variety

35 Syrah an ABA-related stomatal closure was induced in WR soil to maintain high levels

36 of water potential, showing that a soil-related hormonal root-to-shoot signal causing

37 stomatal closure superimposes on the putatively variety-induced anisohydric response to

38 water stress.

39 **Key words:** abscisic acid (ABA), cavitation, embolism, hydraulic conductance, water

40 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

48 grapevine plants, some cultivars have been more frequently observed expressing

49 consistent results than others. One of these is Syrah. This cultivar, of mesic origin, has

50 been mainly categorized as anisohydric, either from observations of plants under field

51 conditions (Schultz 2003; Rogiers *et al.* 2009; Soar *et al.* 2009) or in pots (Soar *et al.*

52 2006). Cabernet Sauvignon, on the other hand, has been more frequently observed to

53 display a response to water deprivation nearer to isohydric type (Hochberg *et al.* 2013).

54 Owing to the differential response observed on these two cultivars under the same water

55 conditions, Cabernet Sauvignon and Syrah have already been coupled in comparative

56 experiments (Chalmers 2007; Petrie and Sadras 2008; Rogiers *et al.* 2009; Hochberg *et*

57 *al.* 2013) and can therefore be selected as efficient models for representing iso- and

58 anisohydric behaviours.

59   The stomatal control, which is an endogenous, but highly variable character, was

60   considered in combination with the soil effect. Soil is in fact another crucial component

61   in grape and wine production, not only because it determines the water and nutrients

62   availability for the plant and therefore its productive performances, but also for its

63   specific implication in the "*terroir* effect" in viticulture (Bodin and Morlat 2006; van

64   Leeuwen *et al.* 2009). In spite of the acknowledged importance on grape and wine

65   production, not many studies attempted to quantify its effects with comparative trials.

66   For this reason, in the presented work, we decided to focus the attention only on the

67   differences produced by two soils in terms of soil texture and related water availability

68   provided to the plant: one single aspect which is, however, strongly influenced by

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).

72   Cavitation of the xylem vessels is a very relevant consequence of the limited soil

73   moisture, as it can produce dramatic consequences by reducing the hydraulic

74   conductivity of the vascular tissues and impairing the possibility for the plant to replace

75   transpired water (Brodersen *et al.* 2013). It is also one of the most studied effects of

76   drought in grapevine, in combination with loss in hydraulic conductance (Lovisolo and

77   Tramontini 2010). In leaves, cavitation and consequent embolism formation affect

78   mainly the leaf midrib (Blackman *et al.* 2010), with a conductivity loss in grapevine

79   petioles of 50% at Ψ_{stem} of -0.95 MPa and of more than 90% at -1.5MPa (Zufferey *et al.*

80   2011). On the other hand, the entity of damage produced by cavitation and the break

81   against its propagation are modulated by the speed and intensity of stomata reaction and

82   by its effect on transpiration (Domec and Johnson 2012) approximating leaves to

83   hydraulic fuses of the plant (Zufferey *et al.* 2011).

84   Embolism formation and repair is controlled by a likely hydraulic mediation at the leaf

85   level (Pantin *et al.* 2013) and via chemical signals (Salleo *et al.* 1996; Lovisolo and

86   Schubert 2006) among which abscisic acid (ABA) has a crucial role. ABA is in fact the

87   hormone devoted to drive the stomatal response to drought: when the soil water

88   potential declines, ABA acts as a messenger indicating water stress from the roots, via

89   the xylem sap, to the guard cells in the leaves and inducing the stomata closure

90   (Hartung *et al.* 2002), limiting in such a way the potential consequences of embolism

91   formation (Chitarra et al. 2014). When the water availability is recovered to an adequate

92   level, the roots stop releasing the hormone and the stomata re-open. The delayed

93   interruption of the signal, much more gradual than the initial release, suggests a further

94   action of the hormone on the embolisms repair (Lovisolo *et al.* 2008; Perrone *et al.*

95   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

102   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.

Materials and Methods

106

Plant material and growing conditions

107

108   The trial was conducted in August 2012 at Hochschule Geisenheim University

109   (Geisenheim, Germany) on 16 three-year-old plants of *Vitis vinifera* L. of two

110   genotypes: 8 plants of 'Cabernet Sauvignon' and 8 of 'Syrah'. Both were grafted on

111   hybrids of *Vitis berlandieri* × *Vitis riparia* ('161-49 Couderc'for 'Cabernet Sauvignon'

112   and '420A Millardet Et De Grasset' for 'Syrah') of comparable characteristics (Whiting

113   2004), especially in controlling the interrelationship between leaf or stem water

114   potential and stomatal conductance (Tramontini *et al.* 2013b). The plants were

115   maintained under glasshouse conditions with no supplementary light or heating in 9 L

116   (24 cm average diameter) plastic pots filled (20 cm depth) with two different substrates,

117   one water draining (WD soil) and the other water retaining (WR soil). The WD

118   substrate was composed of 80 % vol. of sand and 20 % vol. of ED 73 (Einheitserde

119   Classic, Einheitserde-Einheitserde- und Humuswerke Gebr. Patzer GmbH & Co.KG,

120   Sinntal, Germany; consisting of 55% white peat, 30% clay, 15% sod peat; chemical

121   properties pH ($CaCl_2$) 5.8, salt content 2.5 g L^{-1}) including nutrient salt (14+16+18, 1 kg

122   m^{-3}) and a slow-release fertilizer (Gepac LZD 20+10+15, 2 kg m^{-3}), the WR substrate

123   consisted entirely of ED 73.

124   Plants were watered to container capacity at the beginning of the experiment

125   (Tramontini *et al.* 2013b) and fertilized in order to bring them to the same level of

126   nitrogen availability. Soil nitrogen content after the fertilization was estimated

127   according to Robinson recommendations (1988), confirming that at the beginning of the

128   experiment the two different substrates had approximately the same amount of available

129   nitrogen. Data collection started when the plants had reached a mild water stress (Ψ_{stem}

130    $\leq$ -0.5 MPa), such as four days after interruption of irrigation. In that moment plants had

131   14.4 ± 2.8 leaves with no significant differences between cultivars or soils. Each plant

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

135   assessed during the whole duration of the experiment. All measurements were taken

136   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

151   a plastic sheet and covered with aluminium foil to stop transpiration at least 1 h before

152   measurements were taken.

153   *Xylem embolism*

154   Daily determination of xylem embolisms in leaf petioles, induced by the presence of air

155   bubbles in xylem vessels, was carried out around midday using a high-pressure

156   flowmeter (HPFM, Dynamax Inc., Houston, TX, USA) (Tyree *et al.* 1995). As the

157   assessment of embolism extent is a destructive analysis, leaf petioles were used as a

158   proxy of the plant behaviour (Lovisolo *et al.* 2008; Perrone *et al.* 2012). During the

159   whole duration of the experiment macro- and microbubbles were regularly flushed out

160   of the system according to the manufacturer`s instruction manual and the mismatch

161   between the two pressure transducers was controlled daily by running the 'Set Zero'

162   routine before measuring.

163   For each determination of percent loss of conductivity (PLC), the petioles and leaves

164   were cut under water from the shoots and immediately attached to the HPFM tubing

165   under water preventing air bubbles to enter the system. The leaves were cut ~1 cm

166   above the petiole insertion a few seconds after starting the measurement. The initial

167   hydraulic conductance K_{hi} was determined applying an initial pressure of ~20 kPa for 3

168   min. Distilled and degassed water with an addition of 10 mmol L^{-1} KCl was used as

169   perfusion liquid. Petioles were then flushed for 3 min applying a transient increase of

170   pressure until a pressure of ~550 kPa was reached. This pressure was kept constant for 3

171   min. To determine the final hydraulic conductance K_{hf} the pressure was downregulated

172   to ~20 kPa and held constant for 3 min. To calculate K_{hi} and K_{hf} average values of the

173   hydraulic conductance of the respective timespans were used.

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:

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

177

178   After the embolism determination the length and the maximum and minimum diameter

179   of 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

184   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

187   method described by Materán *et al.* (2009) with some adaptations: 2 g of frozen tissue

188   were grounded to powder under liquid nitrogen, 5 ml of 80 % Methanol were added and

189   the samples were extracted at 4 °C overnight. Samples were centrifuged at 4000 rpm for

190   5 min, the supernatant was transferred to a flask and methanol was evaporated. The pH

191   was adjusted to values between 8-9 with a phosphate buffer; 1 ml of ethyl acetate was

192   added and samples were centrifuged at 4000 rpm for 5 min; after discarding the

193   supernatant, the pH was adjusted to 2-3 (with 1N HCl), 2 ml of ethyl acetate were added

194   and the samples were centrifuged at 4000 rpm for 5 min. The supernatant was removed

195   and the ethyl acetate fraction was evaporated. The dry residue was re-suspended in

196   methanol, filtered in brown vials and injected into a 1260 Infinity HPLC-DAD System

197   (Agilent Technologies, Cernusco sul Naviglio, Milano, Italy). ABA was separated on a

198   Purosphere® STAR RP-18, 5 μm, LiChroCART (250-4) (Merck, Darmstadt, Germany)

199   column thermostated at 35 °C. The solvent gradient used was 100 % A (94.9 % H_2O: 5

200   % CH_3CN: 0.1 % HCOOH) to 100 % B (5 % H_2O: 94.9 % CH_3CN: 0.1 % HCOOH)

201   over 20 min. Solvent B was held at 100 % for 10 min then the solvent returned to 100 %

202   A (Forcat *et al.* 2008). The flow rate into the column was set at 0.5 ml/min. DAD

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

205   by building a calibration curve with (±)- Abscisic acid, ≥ 98.5 % (Sigma Aldrich SRL,

206   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

208   the standard solution.

209   *Statistical analysis*

210   Regression coefficients were obtained using Excel (Microsoft, Redmond, WA, USA),

211   and statistical analysis was performed with univariate analysis of variance (ANOVA)

212   and multivariate analysis of variance (MANOVA) to reveal differences among cultivars

213   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**

217   *Interrelationships between stomatal conductance and soil and stem water potential in*

218   *different soils and cultivars*

219   Our observations excluded the initial phase of optimal water availability and focused on

220   the dynamics of water relations evolving from mild (day 1 of measurements) to extreme

221   drought, as shown in Fig. 1. The soil water content between WR and WD soils was very

222   different from the beginning, however, the dynamics of the daily averages of Ψ_{stem} and

223   g_s did not express constant differences between soils and cultivars along the period of

224   the trial. The proportion of embolized vessels at petiole level (PLC) was higher on WD

225   soil than on WR for most of the trial, but not constantly along the trial.

226   In spite of that, the relationship between Ψ_{stem} and θ highlights how the two substrates

227   are distinct for their effect on plant water status (Fig. 2). These differences are already

228   evident at mild water stress conditions (Ψ_{stem} around -0.5 MPa) and while on WR soil

229   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).

233 The measured Ψ_{stem} was then combined with the calculated soil water potential (Ψ_{soil})

234 (Fig. 3). The obtained curves show that during water stress Ψ_{stem} declined following a

235 decrease in Ψ_{soil}. In Cabernet Sauvignon this plant adaptation was evident at mild stress

236 conditions, and apparently delayed (and/or less effective) in Syrah.

237 The response of g_s to Ψ_{stem} was maximum at the beginning of the trial with an overlap

238 of the two curves representing the two cultivars at around -1.4 MPa (Fig. 4a). In

239 comparison to Syrah Cabernet Sauvignon showed lower g_s under mild water stress

240 conditions without strong changes under severe water stress conditions characterising

241 its isohydric behaviour. Our experiment focuses on results obtained under stress, but

242 hypothetical relationships preceding limiting conditions can be drafted: in these

243 conditions Cabernet Sauvignon would probably have shown a steep adaptation to water

244 stress, while Syrah progressively coupled stomatal function with decreasing plant water

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,

247 whereas on WR soil they become minimized. Syrah maintains generally higher g_s

248 values than Cabernet Sauvignon, but, while, at a given Ψ_{stem}, in Syrah g_s 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

251 these differences in g_s of the two cultivars appear significantly valid at Ψ_{stem} not lower

252 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

256 to run a statistical analysis of results collected at comparable level of soil water

257 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

261 conditions, in WR soils a stomatal closure is induced to maintain high levels of stem

262 water potential. In Cabernet Sauvignon the putative isohydric control on water potential

263 is not so effective, as in parallel to a not significant stomatal closure, plants respond to

264 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

266 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,

269 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

271 observed between the two soils are statistically significant ($P < 0.05$) with the vines on

272 WD substrates showing a significantly higher PLC compared to WR substrates at Ψ_{stem}

273 < -1 MPa.

274 The analysis of the ABA content in leaves showed that the relationship between ABA

275 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

277 both varieties, significantly in Syrah, the WR soil induces an increase of ABA content

278 in 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

282 as different in their stomatal response to water stress: Cabernet Sauvignon and Syrah.

283 *Hydraulic control of water stress*

284 Water stress effects were already apparent at mild water stress conditions (Ψ_{stem} around

285 -0.5 MPa), when plants started to experience different shrinking capacities of the two

286 substrates. According to Whitmore and Whalley (2009), in fact, when a shrinking soil

287 dries, as WR substrate of our pots, its degree of saturation is kept small in comparison

288 with a drying rigid soil, such as the WD soil of this experiment (Fig. 1). In WD soils,

289   the matric potential becomes negative much faster, lowering the level of saturation after

290   a much smaller amount of water is removed by roots

291   In addition to the soil effect, with $\Delta\Psi$ between soil and stem higher for Cabernet

292   Sauvignon than for Syrah, the two cultivars expressed a different capacity of water

293   extraction from the substrate (Fig. 3), requiring to the former a higher energy in order to

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).

297   On the other hand, Cabernet Sauvignon would preserve soil moisture more efficiently

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

302   preserve soil moisture, but our pot plants have been uniformed to have not different leaf

303   area. The curves obtained from the four combinations soil/cultivar (Fig. 4*b*) could be

304   thus explained by the fact that in water-stress conditions near-anisohydric varieties do

305   not promptly regulate their stomatal conductance and therefore their transpiration rate

306   (which was the case of WD substrate, Fig. 2). On the contrary, near-isohydric varieties,

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.* 2013*a*), the expression of plant reactions to

311   water stress seems to be buffered on clay soils. This could be due to the higher capacity

312   of this kind of soils to hold water and release it gradually to the plant. It could be

313   hypothesized that WR substrate produces an effect similar to that of clay soil,

314   submitting the potted roots to transient drought conditions (produced by the daily

315   fluctuations of dehydration during the day and rehydration during the night) able to

316   interfere with the physical and hormonal signalling between roots and stem. However,

317   as illustrated in Fig. 5, all these differences in g_s are significantly valid at Ψ_{stem} not

318   lower than -1 MPa. When water stress becomes more severe, stomatal regulation is

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).

322 Due to this, the differences between iso- and anisohydric behaviours are evident

323 between mild and moderate water stress, where the expression of the limitations

324 imposed at stomatal level are maximised. In our results, at these conditions, the average

325 g_s is significantly different between varieties but not between substrates (under each

326 variety), although on WD the differences remain evident. Concerning the consequent

327 risk of cavitation, Syrah on both soils and Cabernet Sauvignon on WD have an increase

328 in embolism formation, expressed in terms of xylem conductivity losses, of 32–36%,

329 moving from $\Psi_{stem} >$ -1 MPa to $\Psi_{stem} <$ -1 MPa. Only Cabernet Sauvignon on WR soil

330 shows higher embolism formation at $\Psi_{stem} >$ -1 MPa than at $\Psi_{stem} <$ -1 MPa. An

331 explanation of this phenomenon would require the support of further data concerning,

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

334 ABA to the differential response of g_s in iso- and anisohydric cultivars.

335 *Abscisic-acid control on stomatal conductance*

336 On the near-isohydric cultivar, Cabernet Sauvignon, expressing very similar level of

337 cavitation on the two soils at $\Psi_{stem} >$ -1 MPa, we could observe a more stable ABA

338 signal, independently from the soil (Fig. 7), similarly to observations by Puértolas *et al.*

339 (2013) using *Phaseolus vulgaris* L. In contrast, in Syrah, showing two levels of

340 cavitation on the two soils both at moderate and at higher stress level, also the curves of

341 ABA concentration in leaves were clearly distinguished, between the leaves of plants on

342 WR soil richer on the hormone than those on WD soil, showing a substrate-dependant

343 ABA concentration, as observed by Dodd *et al.* (2010) on *Helianthus annuus* L. In

344 order to analyze better this result we suggest comparing it with that on Fig. 4b: contrary

345 to initial expectations, Syrah has generally higher g_s on WD than on WR soil, and this

346 may be due to the specific circumstances produced by the WR soil, as above-mentioned,

347 favouring the release of the hormone (ABA) in the leaf. As recently observed by

348 Brodribb and McAdam (2013) on two conifer species, the isohydric stomatal regulation

349 can be identified as an ABA-driven stomatal closure, while the anisohydric is at least

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

352 properties. In Syrah plants potted on WD soil a similar ABA control on stomatal

353 conductance subsists. However, when the anisohydric Syrah grows onto the WR soil, an

354 additional ABA leaf biosynthesis or accumulation is recordable. The WR-induced raise

355 in ABA allows stomatal control limiting the anisohydric response, as it happens when

356 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*

359 Our results are in line with those recently presented by Hochberg *et al.* (2013) on a

360 similar work done on the same two varieties and with the general consideration on the

361 differential photoprotective response to stress in iso- and anisohydric cultivars (Pou *et*

362 *al.* 2012). We would expect that plant productivity of Cabernet Sauvignon, due to the

363 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

366 characteristics of the two genotypes, seem to find coherence: Cabernet Sauvignon, the

367 more isohydric variety, thanks to a tight stomatal control, conserves varietal

368 characteristics on the grape independently from the growing conditions. From a

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

371 differences . Hence, the more anisohydric variety, seems to base its stomatal control

372 more on hydraulic signals. This could be hypothesized as the effect of a higher

373 involvement of long term adaptation mechanisms, such as anatomic modifications, and

374 the development of a product which strongly varies according to the characteristics of

375 the substrate. Both are expressions of the *terroir* concept favouring different

376 components and mechanisms to adapt.

377 Although our results have been obtained on potted plants, where the nature of the

378 substrate and the available volume for root development are a limiting projection of the

379 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

383   product. It is considered extremely capable to express the characteristics of a given

384   *terroir* and, due to that, has been for a long time the world's most widely planted

385   premium red wine grape (Robinson 2006). The anisohydric Syrah, on the other hand, is

386   a very common commercial variety (the world's 7th most grown grape in 2004, still

387   according to Robinson 2006) particularly distributed in warmer regions, from which

388   very diverse wines can be produced.

389   Furthermore, ABA plays a key role by stimulating the activation of the anthocyanin and

390   flavonoids biosynthesis pathway (Davies and Böttcher 2009; Ferrandino and Lovisolo

391   2014). Both, its impact on water relations and on berry metabolism may contribute to a

392   differential berry quality.This hypothesis could represent a relevant topic for further

393   studies in field conditions, where also long terms mechanisms of adaptation and more

394   complex dynamics of hormonal signalling (Dodd 2013) can be observed, and extended

395   to other varieties, considering the main mechanisms involved in the *terroir* expression.

396   **Conclusions**

397   In conclusion, we reported a hydraulic control of stomatal responses at the base of the

398   near-anisohydric Syrah adaptations to water stress, in contrast to an ABA-induced

399   stomatal control in the near-isohydric Cabernet Sauvignon. Also is Syrah, however, the

400   hormone-related response could be effective when soil properties allowed for higher

401   water storage buffering hydraulic adaptations.

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553

554   **Figure legends**

555   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

557   embolisms (PLC, %), during the days of the trial. Measurements were conducted on

558   plants of Cabernet Sauvignon (*circles*) and Syrah (*triangles*) on water draining (WD,

559   *white*) and water retaining (WR, *black*) soils. Means ± std err. *Diamonds* in frame (d)

560   represent the mean value of the day for both cultivars grouped.

561   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

563   draining (WD, *white*) and water retaining (WR, *black*) soils. Arrows on the x axis point

564   to maximum water-holding capacity of the two soils (% water at -0.01 MPa).

565   Figure 3. Relationship between stem water potential (Ψ_{stem}, MPa) and soil water

566   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}

568   was obtained from direct measures while Ψ_{soil} from the derived equations of Ψ_{soil} and θ.

569   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

573   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.

575   Figure 5. Average values of leaf stomatal conductance (g_s, mmol m^{-2} s^{-1}) measured on

576   plants of Cabernet Sauvignon on water retaining soil (WR, *black*) and on water draining

577   soil (WD, *light grey*) and on Syrah plants on WR (*dark grey*) and on WD (*white*). Data

578   have been clustered for those collected between mild and moderate water stress (Ψ_{stem} >

579   -1 MPa) and high water stress (Ψ_{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).

582 Figure 6. Average values of percent loss of conductivity (PLC, %) due to embolism

583 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

586 mild and moderate water stress (Ψ_{stem} > -1 MPa) and high water stress (Ψ_{stem} < -1 MPa).

587 Values of bars topped by common letters are not significantly different, while different

588 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, mmol m^{-2} s^{-1}) and

590 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

592 retaining (WR, *black*) soils. In frame (a), continuous lines represent the two curves

593 obtained for Cabernet Sauvignon and dashed lines for Syrah. In frame (b), means ± std

594 errors are displayed.

595

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.

596

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

599 potential (Ψ$_{soil}$) values: mild (Ψ$_{soil}$ >-0.083), intermediate (-0.083 > Ψ$_{soil}$ > -0.212) and

600 severe water stress (Ψ$_{soil}$ <-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.

603

607

a.

b.

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611