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1 **Short title:** Leaf hydraulic conductance, ABA and isohydry.

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11 **Title:**

Abscisic acid down-regulates hydraulic conductance of grapevine leaves in isohydric genotypes only

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One-sentence summary:

12 Abscisic acid reduces the water transport capacity of grapevine leaves, most notably in isohydric
13 genotypes.

Footnotes:

Authors Contributions:

14 A.C.-L., T.S, S.D.T., A.C. and E.L. designed research; A.C.-L. and D.M. performed the experiments;
15 T.S. and S.D.T. supervised the experiments; E.J.E. supervised ABA analyses; A.C.-L. analysed the
16 data; A.C.-L and T.S. wrote the paper with the contribution of S.D.T.

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21 **Abstract**

22 Plants evolved different strategies to cope with water stress. While isohydric species maintain their
23 midday leaf water potential (Ψ_M) under soil water deficit by closing their stomata, anisohydric species
24 maintain higher stomatal aperture and exhibit substantial reductions in Ψ_M . It was hypothesized that
25 isohydry is related to a locally higher sensitivity of stomata to the drought-hormone abscisic acid
26 (ABA). Interestingly, recent lines of evidence in *Arabidopsis* suggested that stomatal responsiveness is
27 also controlled by an ABA action on leaf water supply upstream from stomata. Here, we tested the
28 possibility in grapevine that different genotypes ranging from near isohydric to more anisohydric may
29 have different sensitivities in these ABA responses. Measurements on whole plants in drought
30 conditions were combined with assays on detached leaves fed with ABA. Two different methods
31 consistently showed that leaf hydraulic conductance (K_{leaf}) was downregulated by exogenous ABA,
32 with strong variations depending on the genotype. Importantly, variation between isohydry and
33 anisohydry correlated with K_{leaf} sensitivity to ABA, with K_{leaf} in the most anisohydric genotypes being
34 unresponsive to the hormone. We propose that observed response of K_{leaf} to ABA may be part of the
35 overall ABA regulation of leaf water status.

36 **Key-words:** Isohydric / anisohydric, ABA, leaf hydraulic conductance, transpiration, stomatal
37 conductance, *Vitis vinifera* L.

38 Introduction

39 Maintaining adequate tissue water content through efficient controls of water supplies and losses is a
40 key requirement for crop performance and plant survival in dry environments. Accordingly, plants
41 evolved with varied capacities to close stomata in response to soil drying, thereby limiting the drop of
42 water potential along the transpiration path yet at the expense of carbon assimilation and growth.
43 Optimization between carbon gain and water loss has resulted in the evolution of a continuum of
44 strategies among species, ranging from isohydry to anisohydry. Anisohydric species exhibit
45 substantial decrease in their midday leaf water potential (Ψ_M) as soil water deficit develops, while
46 isohydric species maintain higher Ψ_M through stomatal closure at incipient stages of soil drying
47 (Tardieu et al., 1996). A wide spectrum of behaviours has also been observed between varieties of the
48 same species, as in apple tree *Malus* (Massonnet et al., 2007) and grapevine *Vitis vinifera* (Schultz,
49 2003; Soar et al., 2006; Prieto et al., 2010). In *V. vinifera*, genomic regions (QTLs) have been
50 identified that control the maintenance of Ψ_M under moderate water deficit (Coupel-Ledru et al.,
51 2014). Although dependent on environmental conditions (Franks et al., 2007), variation from iso- to
52 aniso-hydry has therefore a clear genetic basis.

53 How stomata coordinate with plant hydraulics to optimize Ψ_M in response to drought and how this
54 may vary between species remain a matter of debate. Yet, it has been frequently reported that stomatal
55 response parallels the dynamics of hydraulic conductance in roots (Zufferey and Smart, 2012;
56 Vandeleur et al., 2014), leaves (Cochard et al., 2002) or whole plants (Meinzer, 2002; Zufferey and
57 Smart, 2012). This observation has been mostly interpreted as the result of a biological coupling
58 between water supply (hydraulic conductance) and water demand (transpiration), preventing water
59 potential along the transpiration path from dropping to damaging levels (Cochard et al., 1996; Sack
60 and Holbrook, 2006; Franks et al., 2007; Simonin et al., 2015). On the one hand, water deficit may
61 impact on water supply either via the development of cavitation in xylem conduits (Tyree and Sperry,
62 1989), thereby reducing hydraulic conductance from the inner root tissues to the leaf petiole, or
63 downregulation of aquaporin activity which controls water transfer through membranes of living cells
64 in both roots and leaves (Chaumont and Tyerman, 2014). On the other hand, stomata primarily control
65 the response of transpiration to water deficit. The physiological mechanisms underlying stomatal
66 response most likely involve both hydraulics and biochemistry with the accumulation of the drought
67 hormone abscisic acid (ABA). Water deficit draws down water potential in all plant tissues and may
68 directly impair turgor pressure in the guard cells surrounding the stomatal pores, thus reducing
69 stomatal aperture (Buckley, 2005; Peak and Mott, 2011). In parallel, accumulation of ABA -whether
70 synthesised in roots (Simonneau et al., 1998; Borel et al., 2001) or leaves (Christmann, 2005;
71 Christmann et al., 2007; Ikegami et al., 2009; McAdam et al., 2016)- directly impacts on guard cells to
72 close stomata (Kim et al., 2010; Joshi-Saha et al., 2011).

73 The relative contribution of ABA signalling and hydraulics to drought-induced stomatal closure varies
74 depending on species (Tardieu et al., 1996; Brodribb and Jordan, 2011; Brodribb and McAdam, 2013;
75 McAdam and Brodribb, 2013; Brodribb et al., 2014; McAdam and Brodribb, 2014). Interestingly, leaf
76 water potential appears to sensitize guard cells to the effect of ABA, thus resulting in a feedforward
77 effect on stomatal closure upon water stress (Tardieu and Davies, 1992). Moreover, this feedforward
78 effect is only observed in isohydric species (Tardieu and Simonneau, 1998). Although such apparent
79 interaction between hydraulics and ABA accounts for the distinction between isohydric and
80 anisohydric behaviours, the biological basis for this observation remains unknown.

81 Recent studies on the isohydric species *Arabidopsis* challenged the classical view that ABA induces
82 stomatal closure by solely acting at the guard cell level. First, ABA reduces leaf hydraulic
83 conductance (K_{leaf}) through the downregulation of aquaporin activity in the bundle sheath around leaf
84 veins (Shatil-Cohen et al., 2011). Second, xylem-fed ABA induces parallel reductions in K_{leaf} and
85 stomatal conductance in leaves of mutants that are insensitive to ABA at the guard cell level (Pantin et
86 al., 2013). These results gave rise to the proposal that ABA promotes stomatal closure in a dual way,
87 via its local, biochemical effect on the guard cells, but also via a remote, hydraulic impact of a drop in
88 water permeability within the bundle sheath. Bundle sheath cells were thus assigned a role of ‘control
89 centre’ for water flow, able to convert ABA signalling into feedforward hydraulic signals up to guard
90 cells. We surmised that such hydraulic effect of ABA may underlie the apparent interaction between
91 hydraulics and ABA on stomatal control of isohydric species (Tardieu and Simonneau, 1998), and thus
92 originate the genetic differences between isohydric and anisohydric behaviours.

93 Here, we tested this hypothesis by examining the relationship between K_{leaf} sensitivity to ABA and
94 (an)isohydric behaviour of grapevine genotypes obtained from a cross between two contrasting
95 cultivars, the near-anisohydric Syrah and the near-isohydric Grenache (Schultz, 2003; Soar et al.,
96 2006; Prieto et al., 2010). A wide range of variation for (an)isohydry was evidenced within the
97 offspring, ranking far beyond the parental behaviours (Coupel-Ledru et al., 2014). We selected a panel
98 of contrasting genotypes and combined experiments on whole plants under two watering regimes with
99 measurements on detached leaves fed with various ABA concentrations. The inhibiting effect of ABA
100 on K_{leaf} was only observed in those genotypes that showed strong stomatal closure and typical
101 isohydric behaviour upon water deficit. By contrast, K_{leaf} of more anisohydric genotypes was
102 insensitive to ABA. These results support a major role for genetic variation in K_{leaf} sensitivity to ABA
103 in determining (an)isohydric behaviour in grapevine.

104 **RESULTS**

105 **Two independent methods reveal differential sensitivity of K_{leaf} to ABA between Syrah and** 106 **Grenache**

107 To investigate the putative link between ABA, leaf hydraulics, and plant (an)isohydric behaviour, we
108 assessed the effect of ABA on K_{leaf} on two grapevine cultivars reputed to be isohydric (Grenache) and
109 anisohydric (Syrah). K_{leaf} response to ABA was first characterized using the Evaporative Flux Method
110 (EFM) on detached leaves that were xylem-fed for one hour with a control solution or with a solution
111 of exogenous ABA at either a concentration of 2, 4, 8, 16 or 32 mmol m^{-3} . K_{leaf} displayed a strong
112 sensitivity to ABA in Grenache, declining from 12 $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ in the control solution to 5
113 $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ in the 32 mmol m^{-3} ABA solution (Fig. 1 A). By contrast, K_{leaf} of Syrah was much
114 less sensitive to ABA (Fig. 1 B), with a slight decrease that was not found significant ($p>0.1$).

115 To consolidate these results, we measured K_{leaf} sensitivity to ABA with a second, independent method.
116 We used the High Pressure Flow Meter (HPFM) to measure K_{leaf} in detached leaves of Grenache and
117 Syrah fed for one hour with control or ABA solutions (Fig. 1 C&D). Overall, whatever the method
118 used, K_{leaf} was strongly and significantly reduced by ABA-feeding in the near isohydric cultivar
119 Grenache ($p<0.01$) but not in the near anisohydric one Syrah. K_{leaf} values obtained in control
120 conditions with the HPFM were highly consistent with those previously reported by Pou et al. (2013)
121 who operated similarly. However, K_{leaf} values were three-fold higher when measured with the HPFM
122 as compared to the EFM, which suggests that K_{leaf} might be overestimated by the HPFM because of
123 the higher hydrostatic pressure imposed to water within the leaf (Prado and Maurel, 2013) while
124 negative pressures develops in transpiring leaves. For this reason, the EFM most likely mimicked the
125 natural pathway of water in leaves through the transpiration flow (Sack and Scoffoni, 2012) even
126 though the flow rate was of the same order of magnitude whatever the method used (between 0.5 and
127 $3 \text{ mmol m}^{-2} \text{ s}^{-1}$; see also Fig. S2).

128 The HPFM method also made it possible to distinguish the conductance between petiole and lamina.
129 For that purpose, just after K_{leaf} measurement with the HPFM, the leaf was cut at the junction between
130 petiole and lamina and hydraulic conductance of the petiole (K_{petiole}) was determined. The hydraulic
131 conductance of the lamina (K_{lamina}) could then be derived considering that water pathways in petiole
132 and lamina operate in series. Irrespective of the cultivar, the conductance in the petiole was much
133 stronger (about ten-fold higher) than in the lamina when calculated at the whole organ level, indicating
134 that most of the resistance to water transfer takes place in the lamina. No effect of ABA was observed
135 for Syrah on either part of the leaf (Fig. 1 F&H) as could be expected from the absence of effect on
136 overall K_{leaf} (Fig. 1 D). By contrast, in the nearly isohydric Grenache, ABA feeding markedly reduced
137 K_{lamina} (Fig. 1 G, $p<0.01$) while K_{petiole} was not significantly affected (Fig. 1 E).

138 Measurements at the leaf and the lamina level also revealed a significant difference in K values
139 recorded before ABA perfusion: Grenache displayed higher initial K_{leaf} (and K_{lamina}) than Syrah (Fig.
140 1). K_{leaf} at maximum ABA concentration in Grenache reached about the same value as the initial K_{leaf}
141 in Syrah.

142 Stomatal responses to ABA (measured by porometry) were much closer to each other between Syrah
143 and Grenache (Fig. S1) than differences in K_{leaf} response to ABA that were detected using the HPFM
144 method.

145 **Variability in (an)isohydry and ABA accumulation for ten selected offspring genotypes and the** 146 **parental cultivars**

147 Analysis was then extended to a panel of genotypes with contrasting (an)isohydric behaviours. Ten
148 offspring genotypes were selected in the Syrah \times Grenache population based on the change in midday
149 leaf water potential (Ψ_M) previously observed between well-watered and water-deficit conditions
150 (Coupel-Ledru et al., 2014). Ψ_M measured in the selected genotypes under water deficit and controlled
151 atmospheric conditions ranged from -1.1 to -0.85 MPa (Fig. 2 A). The drop in leaf water potential
152 ($\Delta\Psi_M$) between well-watered and water-deficit regimes displayed a highly significant effect of the
153 genotype ($p < 0.001$), ranging from -0.5 MPa for the most anisohydric genotype to -0.1 MPa for the
154 most isohydric one (Fig. 2 B). Change in Ψ_M induced by drought in the parents was intermediate with
155 a slightly better maintenance in Grenache ($\Delta\Psi_M$ of -0.15 MPa) compared to the more anisohydric
156 Syrah ($\Delta\Psi_M$ of -0.25 MPa).

157 We also assessed the variability in ABA accumulation in response to soil drying. For this purpose,
158 ABA was assayed in xylem sap that was collected on leaves of two intact plants per genotype (10
159 offspring and 2 parental) under soil water deficit (WD) and standardized transpiring conditions.
160 Genotype had a significant effect on ABA concentration in the xylem sap of WD plants ($p < 0.01$).
161 Across genotypes, ABA concentration in the xylem sap ranged from 0.5 to 2.8 mmol m⁻³ (Fig. 3 A).
162 Syrah displayed much lower ABA concentration in the xylem sap (about 0.8 mmol m⁻³) than Grenache
163 (1.9 mmol m⁻³). ABA concentration in the xylem sap did not match with the ranking of genotypes
164 according to (an)isohydry level, and did not correlate with $\Delta\Psi_M$ (Fig. 3 B). This rules out a simple role
165 of genetic variation of ABA accumulation induced by water deficit in the determinism of
166 (an)isohydry. We also examined whether higher ABA content could be responsible for reduced K_{leaf} ,
167 even under well-watered conditions, specifically in those genotypes where K_{leaf} did not respond to
168 further treatment with exogenous ABA. ABA concentration was therefore determined in xylem sap
169 extruded from leaves of Syrah and Grenache grown under well-watered conditions. Average ABA
170 content was lower in Syrah (0.5 ± 0.2 mmol m⁻³, $n=4$) than in Grenache (1.1 ± 0.5 mmol m⁻³, $n=4$).
171 This result rules out a possible role of high initial ABA levels in leaves on the absence of K_{leaf}
172 response for genotypes like Syrah.

173 **Genetic variation in K_{leaf} sensitivity to ABA correlates with the degree of isohydry**

174 K_{leaf} response to xylem-fed exogenous ABA was characterized on detached leaves of the 10 offspring
175 genotypes and the parents using the EFM. In the absence of ABA, genetic variability was observed for

176 K_{leaf} ($p < 0.001$), ranging from 2.5 to 13.5 $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ (Fig. 4, Fig. 5 A). Feeding with ABA
177 solutions at various concentrations had contrasting effects depending on the genotype (Fig. 4).
178 Grenache and Syrah responses were consistent with those reported in the previous experiment (Fig. 1).
179 Most of the change in K_{leaf} was observed for xylem ABA varying between 0 and 5 mmol m^{-3} ,
180 corresponding to actual concentrations reported in the xylem sap of grapevines under various soil
181 water conditions (e.g. Rogiers et al., 2012). Five genotypes exhibited a Grenache-like response to
182 ABA, with a strong, significant ($p < 0.001$) reduction of K_{leaf} when ABA concentration was increased
183 (Fig. 4 A-E). By contrast, the seven other genotypes showed a Syrah-like response, with non-
184 significant effect of ABA on K_{leaf} (Fig. 4 F-L).

185 Linear models were then fitted to semi-logarithmic transformed data (Fig. S3), and K_{leaf} sensitivity to
186 ABA was calculated as the slope of this regression, giving the expected change in K_{leaf} for any e -fold
187 increase in [ABA]. The more negative was the slope, the more sensitive to ABA was K_{leaf} . Sensitivity
188 was thus calculated as the opposite of the slope. Although confidence intervals on slopes were quite
189 large (Fig. 5 B), analysis of covariance revealed a significant effect of the genotype on K_{leaf} sensitivity
190 to ABA ($p < 0.01$). Moreover, K_{leaf} sensitivity to ABA strongly correlated with the initial level of K_{leaf}
191 before ABA inhibition ($K_{\text{leaf max}}$, Fig. 5 C).

192 We next tested the relationship between K_{leaf} sensitivity to ABA and (an)isohydry by examining the
193 correlation with ($\Delta\Psi_M$) for all selected genotypes. The correlation was highly significant and positive
194 (Fig. 5 D). This confirmed that the genotypes with the most sensitive K_{leaf} to ABA had a better
195 capacity to maintain Ψ_M at high level under soil water deficit. By contrast, the genotypes with K_{leaf}
196 being hardly responsive to ABA exhibited a substantial drop in Ψ_M under dry soil conditions.

197 It is somewhat counter-intuitive that a stronger reduction in K_{leaf} may result in a better maintenance of
198 leaf water potential upon water deficit. A drop in leaf hydraulic conductance is rather expected to
199 lower leaf water potential, provided leaf transpiration rate (E_{leaf}) is constant. However, ABA feeding
200 also induced a strong reduction in E_{leaf} in detached leaves (Fig. S4, Fig. S5, Fig. S 6). This result
201 supports the assumption that a drop in K_{leaf} upon ABA increase could be responsible for a substantial
202 stomatal closure which could dominate on moderating the drop in leaf water potential (Fig. S7).

203 **Predicting genotypic response to water deficit from ABA accumulation and sensitivity to ABA**

204 We then examined the hypothesis that genetic variation in K_{leaf} sensitivity to ABA correlated with the
205 reduction of E_{leaf} in intact plants submitted to water deficit. For this purpose, we combined the
206 sensitivities of K_{leaf} and E_{leaf} to ABA, as estimated in detached leaves of each genotype (Fig. 5 B,
207 Fig. S3, Fig. S5 and Fig. S6), with the native ABA concentration that was measured in the xylem sap
208 (Fig. 3 A) of plants under soil water deficit. In addition, E_{leaf} and K_{leaf} of well-watered plants were
209 estimated as the maximum values observed in detached leaves fed with ABA-free solution assuming

210 that they were representative of leaves attached on well-watered plants. Changes in E_{leaf} and K_{leaf}
211 induced by water deficit were then related to these maximum values determined for each genotype,
212 yielding % reduction in leaf hydraulic conductance (% reduction K_{leaf}) and in transpiration rate (%
213 reduction E_{leaf}).

214 The correlation between % reduction E_{leaf} and % reduction K_{leaf} could then be examined (Fig. 6 A).
215 Overall, E_{leaf} predicted from ABA concentrations showed higher sensitivity to water deficit than K_{leaf} ,
216 yet the predicted changes in E_{leaf} (% reduction E_{leaf}) covered a smaller range of genetic variation within
217 the panel of genotypes (between 38% for the less responsive genotype and 50%, Fig. 6 A) compared
218 to the genetic range observed for the % reduction K_{leaf} (between 0 and 38%, Fig. 6 A). Despite this
219 difference, % reduction E_{leaf} positively correlated with % reduction K_{leaf} (Fig. 6 A). Similar results
220 were obtained when plotting % reduction K_{leaf} against relative changes in transpiration rate directly
221 measured on the whole plants (Fig. 6 B) during the high-throughput experiment (% reduction E_{plant}).
222 This suggested that the more K_{leaf} was reduced by ABA accumulation under water deficit, the more
223 E_{leaf} was reduced. In agreement with our initial assumption, a better maintenance of plant water
224 potential could be expected for those genotypes with K_{leaf} and thus E_{leaf} more sensitive to ABA.

225 DISCUSSION

226 This study demonstrates that ABA may downregulate K_{leaf} in grapevine with a variable effect
227 depending on the genotype. Previous works reported a role for ABA on plant hydraulics in
228 *Arabidopsis thaliana* by means of mutants (Shatil-Cohen et al., 2011; Pantin et al., 2013). Here, we
229 used two grapevine cultivars contrasting for their water use strategies in drought conditions (i.e. Syrah
230 and Grenache), plus ten offspring from a population obtained from their cross (Coupel-Ledru et al.,
231 2014). Natural variations for K_{leaf} sensitivity to ABA could thus be detected within a species largely
232 cultivated in drought-prone areas (Schultz, 2000; Jones et al., 2005). We observed that genetic
233 variation in the sensitivity of K_{leaf} to ABA correlated with variation in (an)isohydric behaviour in
234 grapevine. We propose that the dual effect of ABA on stomata, via its direct biochemical effect on
235 guard cells and the indirect consequence of K_{leaf} downregulation on guard cell turgor, may underlie
236 part of this genetic variation.

237 **Differential K_{leaf} sensitivity to ABA: a physiological process involved in the variability of plant** 238 **response to drought**

239 In our study, genetic variation was found for ABA-induced responses to drought at two levels: (i)
240 ABA accumulation in the xylem sap of intact plants exposed to drought, and (ii) leaf sensitivity to the
241 hormone by using detached leaves. On the one hand, ABA accumulation in the xylem sap of intact
242 grapevine plants was highly dependent on the genotype, suggesting that ABA biosynthesis capacity or
243 catabolism varied across genotypes. These variations may be due to differential expression of genes

244 associated with ABA synthesis, such as *NCED1* and *NCED2*, or genes coding for enzymes involved in
245 ABA degradation to inactive compounds, including the ABA 8'-hydroxylases (Riahi et al., 2013;
246 Speirs et al., 2013). On the other hand, exogenous ABA application to detached leaves using high
247 concentration (32 mmol m^{-3} (+)-ABA) reduced K_{leaf} by up to 50% for the most sensitive grapevine
248 genotype (Fig. 4). This reduction in K_{leaf} is comparable to that observed for similarly high ABA
249 concentrations on *Arabidopsis* leaves (Pantin et al., 2013). A plateau was observed at the highest
250 concentrations indicating that maximal effect of the hormone on K_{leaf} was reached at ABA
251 concentration far above the physiological range observed even under severe drought (Rogiers et al.,
252 2012). Most importantly, a strong variability in K_{leaf} sensitivity to ABA was observed between
253 genotypes. Combining these values of K_{leaf} sensitivity to ABA as observed on detached leaves with
254 native ABA concentration made it possible to predict changes in leaf hydraulic conductance
255 (% reduction K_{leaf}) induced for each genotype by the water deficit scenario. Overall, genetic
256 differences in K_{leaf} response to drought (% reduction K_{leaf}) were much more influenced by differences
257 in sensitivity of K_{leaf} to ABA than in ABA accumulation. A wide range of genetic variation was
258 obtained for drought-induced drop in K_{leaf} , going from no reduction in the less sensitive genotypes to a
259 decline by up to 38% for the most responsive ones under the moderate water deficit (Fig. 6). The
260 initial, endogenous ABA content (prior to exogenous ABA feeding) was lower in Syrah than in
261 Grenache -respectively $0.5 \pm 0.2 \text{ mmol m}^{-3}$ and $1.1 \pm 0.5 \text{ mmol m}^{-3}$ -, while Syrah was less sensitive to
262 addition of exogenous ABA than Grenache. This rules out a possible role of initial ABA concentration
263 as responsible for a basal downregulation of K_{leaf} in the less sensitive genotype Syrah. The range of
264 K_{leaf} sensitivities observed within the panel of genotypes significantly correlated with their capacities
265 to maintain leaf water potential in conditions of water deficit (Fig. 5 D). The more sensitive was K_{leaf}
266 to ABA, the better water potential was maintained in leaves under water deficit conditions, i.e., the
267 more isohydric was the genotype. By contrast, in more anisohydric genotypes K_{leaf} was hardly
268 sensitive to ABA. Although variation from iso- to aniso-hydric has been shown to depend on
269 environmental conditions, making their genetic origin debated (e.g. Franks et al., 2007), the genetic
270 contrast between Syrah and Grenache was consistently reinforced across three independent
271 experiments in our study (on whole potted plants in greenhouse, on leaves detached from plants
272 cultivated in the vineyard, and leaves detached from potted plants cultivated outdoors).

273 Assuming that the ABA-induced reduction in K_{leaf} was stronger in isohydric than anisohydric
274 genotypes, it was possible to generate the different behaviours *in silico* by amending a simple model
275 of leaf hydraulics with the observed effect of ABA on K_{leaf} (see Methods and Supplemental method
276 S8, S9 and S10). The model predicted that the water potential of guard cells decreased more rapidly
277 when K_{leaf} was more responsive to ABA, leading to stomatal closure and maintenance of bulk leaf
278 water potential. Figure 7 presents the results of two simulations during progressive soil drying, where
279 hypothetical, isohydric and anisohydric genotypes were built with all parameters maintained identical

280 apart from K_{leaf} sensitivity to ABA, based on observed values within the whole set of 12 genotypes.
281 The simulations were run with the most extreme values combining the highest sensitivity observed and
282 the widest range of variation for K_{leaf} between min and max values. They confirm that differential
283 down-regulation of K_{leaf} by ABA can account for part of the contrasted (an)isohydric behaviours,
284 where genotypes with the most responsive K_{leaf} to ABA better control their transpiration rate, thus
285 reducing the drop in leaf water potential under drought corresponding to an isohydric behaviour.

286 Stronger downregulation of K_{leaf} by ABA occurred in those cultivars with higher, maximal leaf
287 hydraulic conductance ($K_{\text{leaf max}}$; Fig. 5 C). Genetic variation in $K_{\text{leaf max}}$ may arise from difference in
288 intrinsic activity of aquaporins among genotypes or from variation in the vascular relative to the
289 transmembrane, extra vascular water pathways. Multiple leaf and vein traits can influence K_{leaf} (Sack
290 and Scoffoni, 2013). As an example, larger conduit lumens might provide greater xylem conductivity
291 and K_{leaf} , while the whole vein diameters also promote differences in transport capacity when they
292 contain greater sizes and numbers of xylem (Russin and Evert, 1985; Coomes et al., 2008; Taneda and
293 Terashima, 2012). Assuming that ABA downregulates K_{leaf} by lowering aquaporin activity, those
294 genotypes with higher aquaporin activity or greater proportion of extra-vascular pathways would
295 consistently display higher K_{leaf} sensitivity to ABA.

296 Other processes which occur during dehydration may modulate the responses of K_{leaf} to ABA and
297 create genetic variability. Several factors result in a strong decline of K_{leaf} during drought, including
298 cavitation, collapse of xylem conduits and loss of permeability in the extra-xylem tissues due to
299 mesophyll and bundle sheath cell shrinkage (Trifilò et al., 2003; Cochard et al., 2004; Blackman et al.,
300 2010). Xylem resistance to cavitation has been shown to vary in a series a conifer species and to
301 correlate with prolonged stomatal opening after a period of 30 days without water (Brodribb et al.,
302 2014). This suggests that variation between anisohydric and isohydric behaviours may also be ruled by
303 differences in xylem resistance to cavitation. Cavitation unlikely occurred here on detached leaves
304 which had their petiole immersed in water. Endogenous ABA which accumulates in leaves under
305 water stress may have different effects from the one observed in our study with exogenous ABA fed to
306 leaves of irrigated plants. Embolism repair, triggered by ABA, does not apply for water-fed leaves of
307 irrigated plants but could occur in leaves of plants under water deficit (Kaldenhoff et al., 2008;
308 Perrone et al., 2012). This possible action of ABA may introduce some discrepancies between what
309 we observed in leaves detached from irrigated plants and what governs leaf hydraulics and indirectly
310 influences stomatal response in plants under water deficit. Other discrepancies may originate in up-
311 regulation of certain aquaporin gene expression when ABA accumulates in leaves under water deficit
312 conditions (Kaldenhoff et al., 1996). Overall, ABA-triggered decrease in K_{leaf} , as described in our
313 work for early stages of soil drying may combine with further decrease in K_{leaf} due to cavitation under
314 more severe drought, with possible overlapping of mechanisms. This may explain why the genetic

315 variation in K_{leaf} sensitivity to ABA as determined on detached leaves did not strictly correlate with the
316 genetic variation in (an)isohydry as determined on whole plants under water deficit.

317 **The variable sensitivity of K_{leaf} to ABA was confirmed by two independent measurement**
318 **methods and two independent experiments.**

319 Measuring K_{leaf} with the Evaporative Flux Method as the flow rate (i.e. transpiration rate E_{leaf}) divided
320 by the leaf water potential (Sack, 2002) has long been a matter of debate when exploring the
321 relationship between E_{leaf} and K_{leaf} (Flexas et al., 2013). By contrast with other methods which use
322 pressurized water to force water flow through the leaf, the EFM respects the native paths of water flow
323 together with the range of negative values for water potentials within the leaf (Sack and Scoffoni,
324 2012). However, calculation of K_{leaf} rests on E_{leaf} which causes some partial correlation between
325 variables and hinders the analysis of their relationship. A second method was therefore used in an
326 independent experiment to provide alternative evidence of ABA acting as a regulator of K_{leaf} . We
327 reiterated our ABA-feeding experiment on detached leaves of the two parental cultivars, Syrah and
328 Grenache, and measured K_{leaf} with the HPFM. This method forces water flow through the leaf by
329 pushing water out of the lamina with a controlled pressure gradient across the leaf (Sack, 2002). K_{leaf}
330 values that were obtained in control conditions with this second method were highly consistent with
331 those previously reported by Pou et al. (2013) who operated similarly (Syrah displayed K_{leaf} of about
332 $20 \text{ mmol m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$ in both studies). However, K_{leaf} measured with the HPFM method was about
333 three-fold higher than the values we obtained for the same cultivars with the EFM (Fig. 1). K_{leaf} might
334 be overestimated by the HPFM because of the higher hydrostatic pressure imposed to water within the
335 leaf while negative pressures develop in transpiring leaves. The leaf is thus flooded with a liquid
336 solution and leaf airspaces might rapidly become infiltrated, implying novel pathway for water
337 movement, hence yielding K_{leaf} values not reflecting *in vivo* context (Prado and Maurel, 2013). By
338 contrast, the EFM was proposed to more closely follow the natural pathway of water in leaves through
339 the transpiration flow (Sack and Scoffoni, 2012). Despite differences in K_{leaf} magnitude, both methods
340 and both independent experiments consistently evidenced a marked difference in K_{leaf} measured in
341 control conditions between cultivars. Importantly, the HPFM allowed uncoupling K_{leaf} response to
342 ABA from E_{leaf} response: because the leaf is flooded in water during the experiment, transpiration rate
343 is non-existent, thus the observed effect of ABA is exclusively a direct one on K_{leaf} . ABA feeding
344 similarly impacted K_{leaf} whatever the method used. The high repeatability across methods and
345 experiments observed for the variable response of K_{leaf} to ABA among cultivars thus strengthens the
346 novel outputs of this work.

347 The use of the HPFM method made it possible to dissociate the effects of ABA on both components of
348 leaf hydraulic conductance, i.e. the lamina and the petiole. Differences in petiole hydraulic
349 conductance between grapevine cultivars have been proposed as a cause of differences in

350 (an)isohydric behaviours between cultivars, although variation with drought was not mentioned
351 (Schultz, 2003). In our study, K_{petiole} was slightly (although not significantly) reduced by the ABA
352 treatment in Grenache whereas it was not in Syrah. Although this result merits further verification, it is
353 consistent with a possible role of the petiole in causing differences in hydraulic behaviours between
354 Syrah and Grenache. Regulation of hydraulic conductance in petioles likely rests on similar
355 mechanism as in the lamina since substantial expression of plasma membrane aquaporins in petioles
356 have been reported (Baiges et al., 2001; Chen et al., 2008), where they may facilitate transcellular
357 water transport. However, the major resistance to water resides in the lamina, so that the petiole may
358 only play a marginal role. By contrast, K_{lamina} offers multiple sites of regulation from the petiole-leaf
359 junction to the sites of evaporation, through apoplasm and symplasm pathways. A significant
360 reduction of K_{lamina} by ABA feeding was observed for Grenache, but not in Syrah (Fig. 1). We are now
361 seeking the mechanism underlying these contrasting behaviours, and good candidates are aquaporins.

362 **Towards the understanding of ABA action on leaf hydraulic conductance**

363 By which mechanism could ABA differentially affect K_{leaf} between genotypes or species remains a
364 key question. Based on the major role of aquaporins in the water permeability of the bundle sheath
365 cells, Shatil-Cohen et al. (2011) suggested that the ABA-induced decrease in K_{leaf} may occur via the
366 downregulation of aquaporin activity therein. Further work showed that silencing a family of plasma
367 membrane intrinsic proteins (PIPs) specifically in the bundle sheath decreases K_{leaf} by a factor of three
368 (Sade et al., 2014). However, experimental evidence is still missing that synchronously links ABA to
369 the macroscopic regulation of K_{leaf} and to the cellular-level regulation of aquaporins. Different studies
370 tried to ascribe physiological responses to water deficit with expression profile of aquaporins, but
371 contrasting results have been obtained depending on both intensity and dynamics of water deficit
372 (Tyerman et al., 2002; Galmés et al., 2007; Prado and Maurel, 2013). Pou et al. (2013) reported lower
373 expression of aquaporin genes *VvTIP2;1* and *VvPIP2;1* and reduced activity of aquaporins in leaves
374 under water deficit coinciding with a decrease of K_{leaf} . Decline of K_{leaf} in dehydrating leaves could also
375 be correlated with low aquaporin activity in the mesophyll (Kim and Steudle, 2007). Importantly,
376 ABA treatment could decrease C-terminal phosphorylation and thus activity of aquaporin AtPIP2;1
377 within 30 minutes of application in *Arabidopsis thaliana* seedlings (Kline et al., 2010). This timescale
378 is compatible with the ABA-induced decrease in K_{leaf} described in our study like in previous works
379 (Shatil-Cohen et al., 2011; Pantin et al., 2013). Altered phosphorylation of aquaporins may act on their
380 trafficking and gating (Törnroth-Horsefield et al., 2006; Prak et al., 2008; Eto et al., 2010) to adjust
381 leaf hydraulics in response to drought, as was described under changing light (Prado et al., 2013) and
382 upon exposure to ABA in guard cells (Grondin et al., 2015).

383 Other proposals have arisen that may explain the contrasts between iso- and anisohydry. Vandeleur et
384 al. (2009) showed that Grenache strongly reduced root hydraulic conductivity under drought

385 contrasting with the more anisohydric Chardonnay, and that this difference was reflected in different
386 responses to drought in transcript abundance of PIP1;1 aquaporin. Whether this relationship was due
387 to changes in water transport and aquaporin expression in roots only or to concomitant changes in
388 leaves as evidenced in our study remains to be elucidated. Such an action of ABA on root hydraulic
389 conductance could have been implemented in our model instead of the direct effect of ABA on K_{leaf} , to
390 yield similar results. Thus, these different mechanisms still have to be deciphered together with their
391 respective importance in the determinism of iso- anisohydry

392 Contrary to most observations in leaves, ABA tends to increase hydraulic conductivity in roots
393 (Ludewig et al., 1988; Zhang et al., 1995; Hose et al., 2000; Thompson et al., 2007). In spite of
394 opposite response to ABA, change in root hydraulic conductivity remains consistent with a role of
395 aquaporins which are downregulated at transcriptional and post-transcriptional levels when ABA
396 concentration increases (Wan et al., 2004; Zhu, 2005; Parent et al., 2009). Opposite reactions in roots
397 and leaves could be associated with selective action of ABA on specific members of the aquaporin
398 family, in order to alleviate the effects of water stress.

399 CONCLUSION

400 This study reveals a substantial genetic variation in the responsiveness of leaf hydraulic conductance
401 to ABA and supports that it may impact isohydry in grapevine. Further studies will inform us whether
402 this relationship is conserved in other species, and whether genetic variations in aquaporin regulation
403 are responsible for the existing variability in isohydry.

404 MATERIALS AND METHODS

405 **Assessing the response to exogenous ABA feeding of the cultivars Grenache and Syrah** 406 **cultivated in the field using two independent methods**

407 *Plant material and preparation*

408 This experiment was performed on Syrah and Grenache plants from the Coombe vineyard (Waite
409 Campus, Adelaide, South Australia). Leaves were prepared as previously described in Pou et al.
410 (2013). Briefly, shoots with mature leaves from the most exposed branches were collected on 5 to 10
411 plants per cultivar at the beginning of the night preceding measurements. Immediately after cutting,
412 shoots were placed into a bucket with their cut ends immersed in distilled water, covered with black
413 plastic bags and taken to the laboratory. The shoots were then re-cut under degassed water and
414 rehydrated overnight in full darkness until leaves were assigned to the perfusion solutions.

415 *Response of transpiration rate on detached leaves of Grenache and Syrah cultivars cultivated in the* 416 *field and determination of K_{leaf} using the Evaporative Flux Method*

417 Mature leaves were chosen on similar position from the apex (8th -12th phytomers). On the morning
418 preceding measurements, leaves were excised from shoots and their petioles were immediately
419 immersed and recut in individual 5 mL containers filled with a filtered (0.2 μm), degassed control
420 solution [2 mol m⁻³ KH₂PO₄, 1 mol m⁻³ MES, 0.4 mol m⁻³ Ca(NO₃)₂] adjusted to pH 6.5. A light
421 source was suspended above the leaves providing ~400 μmol m⁻² s⁻¹ photosynthetically active
422 radiation (PAR) at leaf level.

423 Petioles were tightly sealed to the containers caps. As a precaution, initial transpiration rate was
424 determined on each leaf by weighing leaves in their container every 20 min over a 1h30 time period.
425 Measurements were stopped and discarded if the flow suddenly began to decline, likely due to
426 blockage of water flow in the petiole by residual air bubbles.

427 Abscisic acid (synthetic (±)-ABA), solubilized with a negligible volume of ethanol, was then added to
428 the solution to reach varying concentrations of (+)-ABA (2, 4, 8, 16 or 32 mmol m⁻³). Final
429 transpiration in ABA was determined once the weight declined at a stabilized rate (which occurred
430 about one hour after adding ABA).

431 At the end of the experiment, each leaf was taken off its container and immediately set into a
432 Schölander pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA) to measure
433 its water potential (Ψ_{leaf}). Measurement of leaf water potential with the pressure chamber was assumed
434 to be close to the value for the transpiring leaf just before it is enclosed in the chamber, according to
435 the principle basis of the Schölander chamber. Leaf hydraulic conductance (K_{leaf}) was then calculated
436 following the evaporative flux method (EFM) as the flow rate divided by the leaf water potential taken
437 as the driving force for water flow from the solution (Sack, 2002). The transpiration rate for this
438 calculation was determined as the stable rate of weight loss measured at the very end of the
439 experiment, just before measuring Ψ_{leaf} . K_{leaf} was estimated on a leaf area basis as:

$$440 \quad K_{\text{leaf}} = E / (\Psi_{\text{leaf}} \times LA)$$

441 where LA is the individual leaf area (m²).

442 At the end of measurement, the leaf was scanned and leaf area was determined on photographs using
443 ImageJ (Rasband WS, 2009).

444 ***Response of stomatal conductance on detached leaves of Grenache and Syrah cultivars cultivated in*** 445 ***the field and determination of K_{leaf} using High Pressure Flow Meter***

446 Another set of leaves were chosen and excised following the same protocol as described above. Each
447 leaf was directly assigned to one of the ABA or control solutions (concentration of (+)-ABA 0, 2, 4, 8,
448 16 or 32 mmol m⁻³) prepared as above.

449 After one hour in perfusion, stomatal conductance (g_s) was measured using a porometer (Delta T AP4;
450 Delta-T Devices Ltd, Cambridge, UK) on each leaf fed with a different solution.

451 Leaf hydraulic conductance was then measured with a HPFM apparatus (HPFM-Gen3, Dynamax,
452 Huston USA). This method first developed by Tyree (2005) relies on pushing a solution into a plant
453 part (here, a leaf) at a known delivery pressure. Measurements were performed using the transient
454 method consisting in applying different pressures and recording flow rates to calculate the
455 conductance as the slope of the regression line between flow rate and pressure (Sack, 2002). Leaves
456 were attached to the flow meter through the petiole using compression fittings. Filtered watered was
457 forced into the leaves at increasing pressure (P) up to 0.4 MPa, while measuring the instantaneous
458 flow rate (F) every 2 s (Fig. S2). Corresponding hydraulic conductances (K_{leaf}) were computed from
459 the slope of the plot water flux versus pressure as:

$$460 \quad K_{\text{leaf}} = \Delta F / (\Delta P \times LA)$$

461 where LA is the leaf area (m^2).

462 The lamina was then removed by excising the leaf at the junction with the petiole and the leaf-specific
463 petiole conductivity (K_{petiole}) was measured and computed according to (Sack, 2002) as:

$$464 \quad K_{\text{petiole}} = \Delta F / (\Delta P \times LA \times \text{Petiole length})$$

465 K_{lamina} was then calculated from K_{leaf} and K_{petiole} according to the Ohm's law analogy considering
466 petiole's and lamina's pathways in series: $1/K_{\text{leaf}} = 1/(K_{\text{petiole}} \times \text{Petiole length}) + 1/K_{\text{lamina}}$.

467 During HPFM measurements, the leaves were submerged in a container filled with water in order to
468 maintain constant leaf temperature and prevent transpiration. The temperature in the compartment was
469 adjusted to 23°C with a regulated bath (Ministat, Peter Huber Kälte maschinenbau GmbH, Germany)
470 and continuously aerated. Besides, the HPFM apparatus corrects the hydraulic conductance for
471 possible changes in temperature to account for corresponding changes in water viscosity. The leaves
472 were exposed to the same light as the one used during perfusion.

473 At the end of measurement, the leaf was scanned and leaf area was determined on photographs using
474 ImageJ (Rasband WS, 2009).

475

476 **Experiments on 10 grapevine offspring, and the parents, from a Syrah×Grenache mapping**
477 **population with contrasting (an)isohydric behaviours**

478 *Plant material and preparation*

479 A panel of 10 offspring was selected within the pseudo-F1 population of 186 two year-old genotypes
480 obtained as the first generation from a reciprocal cross between the grapevine cultivars Syrah and
481 Grenache (Adam-Blondon et al., 2004; Coupel-Ledru et al., 2014; Coupel-Ledru et al., 2016).
482 Offspring plus the two parental genotypes were grafted on 110 Richter rootstocks in 2010 and grown
483 outside in 3 L pots for 2 years in Montpellier (France).

484 In winter 2012 (January), plants were transferred to greenhouse in 9 L pots filled with a substrate
485 calibrated for soil water management (Coupel-Ledru et al., 2014). In early spring 2012, the whole
486 population was installed into a high throughput phenotyping platform (PHENOARCH platform hosted
487 at the M3P, Montpellier Plant Phenotyping Platforms, <http://bioweb.supagro.inra.fr/phenoarch>) in
488 another greenhouse where two soil water conditions were applied using automated weighing stations
489 and daily watering. Well-watered conditions (WW), corresponding to a soil water content of 1.5 g
490 water g⁻¹ dry soil, were imposed to half the plants, while the other half was submitted to a moderate
491 soil water deficit (1.05 g water g⁻¹ dry soil). Detailed information of PHENOARCH platform and
492 measurement of environmental conditions is described in (Cabrera-Bosquet et al., 2016).

493 At the end of the high-throughput experiment in the greenhouse, the plants were transferred outside
494 where they were grown for one additional year and pruned to produce one, unbranched leafy axis with
495 their inflorescences removed. Automated ferti-irrigation completed by occasional, individual weighing
496 and watering ensured that all the plants were maintained well-watered (Coupel-Ledru et al., 2014).
497 These plants were used in 2013 for experiments on detached leaves.

498 ***Leaf water potential and transpiration rate of whole plants under controlled conditions in the***
499 ***phenotyping platform***

500 Potted plants were characterized for water relations under transpiring conditions during a 24-h cycle
501 using a controlled environment chamber close to the phenotyping platform. For each genotype, leaf
502 water potential was measured in the daytime (Ψ_M) on three plants per watering regime, using a
503 pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA) (Coupel-Ledru et al.,
504 2014). Isohydic (respectively anisohydic) behaviour was defined as the capacity (respectively
505 inability) of a genotype to maintain leaf water potential in the daytime under water stress. The panel of
506 12 genotypes (ten offspring plus the two parents Syrah and Grenache) was selected based on their
507 contrasted behaviours for (an)isohydry.

508 Transpiration rate was determined in parallel in the same transpiring conditions and as was previously
509 described (Coupel-Ledru et al., 2014; Coupel-Ledru et al., 2016). Each pot was weighed with 0.1 g
510 accuracy (Sartorius balance, IB 34 EDEP, Gottingen, Germany) at the beginning and end of the light
511 period in the controlled-environment chamber. Weight losses were used together with the estimated
512 whole plant leaf area to calculate average transpiration rate on a leaf area basis (E_{Plant}).

513 ***Leaf area***

514 Individual whole plant leaf area (LA) was estimated through processed images taken every two days
515 in the platform imaging cabin as previously described (Coupel-Ledru et al., 2014).

516 ***ABA sampling in the phenotyping platform and quantification***

517 For each of the 12 genotypes (10 offspring plus 2 parental), xylem sap was extracted from two leaves
518 on two WD plants following measurement of Ψ_M with the pressure chamber. The same pressure
519 chamber was used to pressurize the leaf at about 0.3 MPa above the balancing pressure that was
520 imposed for Ψ_M measurement and about 30 μL of sap was expressed from the cut petiole exposed
521 outside the pressure chamber. Expressed sap was collected in 0.5 mL microtubes and conserved at -80
522 $^{\circ}\text{C}$ in a dedicated deep freezer (Herafreeze, HFU T series, Thermo Fisher Scientific, Asheville, NC
523 USA) pending freeze-drying. Microtubes containing frozen sap samples were centrifuged in a
524 centrifuge (Speed Vac Plus SC110A, Fisher Scientific, Illkirch-France) connected to a benchtop
525 freeze-drier (Christ Alpha2-4, Fisher Scientific, Illkirch-France) which imposed a partial vacuum of
526 0.020 mbar. The water contained in the sap samples was sublimated and trapped on the cold condenser
527 of the freeze-drier maintained at a temperature of -80 $^{\circ}\text{C}$.

528 Analysis of ABA abundance in xylem sap was undertaken by liquid chromatography/mass
529 spectrometry (LC MS/MS, Agilent 6410). Dried xylem sap samples were dissolved in 30 μL 10%
530 acetonitrile containing 0.05% acetic acid plus a deuterated internal standard mix (containing D6–
531 3',5',5',7',7',7'-ABA at a concentration of 10 ng mL^{-1}) before introduction into LC MS/MS.
532 Separation was carried out on a C18 column (Phenomenex C18(2) 75mm \times 4.5mm \times 5 μm) at 40 $^{\circ}\text{C}$.
533 Solvents were nanopure water and acetonitrile, both with 0.05% acetic acid. Samples were eluted with
534 a linear 15min gradient starting at 10% acetonitrile and ending at 90% acetonitrile. Compounds were
535 identified by retention times and multiple reaction monitoring. Parent and product ions were the same
536 as previously described (Speirs et al., 2013).

537 ***Response of transpiration rate on detached leaves of the 10 offspring plus 2 parental genotypes***
538 ***under controlled conditions and determination of K_{leaf} using the Evaporative Flux Method***

539 The potted plants used in the whole-plant experiment described above were further cultivated
540 outdoors. In July 2013, one night prior to experiments, plants were transferred from outside to a
541 controlled environment chamber in order to ensure initial, low-transpiring conditions. On the morning
542 preceding measurements, leaves were excised from plants and their petioles were immediately
543 immersed and recut in individual 5 mL containers filled with a filtered (0.2 μm), degassed control
544 solution [2 mol m^{-3} KH_2PO_4 , 1 mol m^{-3} MES, 0.4 mol m^{-3} $\text{Ca}(\text{NO}_3)_2$] adjusted to pH 6.5. Petioles were
545 tightly sealed to the containers caps. Each leaf in its container was then placed in the chamber with

546 VPD maintained at 2 ± 0.2 kPa and temperature at 27°C. Light was provided in the chamber by a bank
547 of sodium lamps that maintained the PPFD at $\sim 480 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the leaf level. All leaves were
548 chosen as well-exposed, fully expanded, generally on the eighth phytomer from the apex.

549 The protocol then followed for ABA perfusion, determination of transpiration rate, of leaf hydraulic
550 conductance with the EFM method and of their response to ABA, was the same as described in the
551 section “*Response of stomatal conductance on detached leaves of Grenache and Syrah cultivars*
552 *cultivated in the field and determination of K_{leaf} using High Pressure Flow Meter*”.

553 **Statistical analyses**

554 Statistical analyses were performed with R (R Development Core Team, 2012). The Kruskal-Wallis
555 test at the 5% alpha level was used for comparison of means. Semi-ln transformations were used to fit
556 linear models to K_{leaf} response to ABA and extract the slopes. Regression slopes were compared by
557 analysis of covariance. Tests for significant differences among all pairs of slopes were further
558 achieved using R compSlopes package with the FDR correction method to adjust p-values and protect
559 against false-positive results.

560 **Modelling the possible influence of downregulation of K_{leaf} by ABA on (an)isohydric behaviour**

561 Two simulations were performed with all parameters maintained identical in both cases apart from
562 K_{leaf} sensitivity to ABA and $K_{\text{leaf max}}$, values of which were chosen based on results obtained on
563 detached leaves of representative isohydric and anisohydric genotypes. We used the most extreme
564 values combining the highest sensitivity observed for genotype 8S034 in Fig. 4 and the widest range
565 of variation for K_{leaf} between min (observed for 7G082 in Fig. 4) and max values (observed for 8S034
566 in Fig. 4). The consequences of a progressive soil drying, characterized by a drop of predawn leaf
567 water potential (Ψ_{pd} , a proxy for Ψ_{soil}) were modelled using Ohm’s law analogy for water transfer from
568 soil to leaves with constant K_{plant} and from leaf xylem to guard cells with K_{leaf} sensitivity to ABA
569 differing between the two simulations. Stomatal response to ABA and guard cell water potential
570 ($\Psi_{\text{guard cell}}$) following equations derived from the Tardieu-Davies model (Tardieu et al., 2015). Full
571 description of the model, equations and parameters initialization is provided in Supplementary method
572 S8, S9 and S10. The model was implemented in R programming language.

573 **Supplemental Material Titles**

574 **Supplemental figure S1.** Response of stomatal conductance (g_s) to ABA for the parental cultivars
575 Syrah and Grenache.

576 **Supplemental figure S2.** Example of raw plots from the HPFM experiments.

577 **Supplemental figure S3.** Data showing effect of exogenous ABA on hydraulic conductance of
578 detached leaves (K_{leaf}).

579 **Supplemental figure S4.** Response of transpiration rate (E_{leaf}) to exogenous ABA measured on
580 detached leaves for the 10 grapevines offspring genotypes and the parents.

581 **Supplemental figure S5.** Transpiration rate of detached leaves (E_{leaf}) as a function of $\ln([\text{ABA}])$ in the
582 feeding sap.

583 **Supplemental figure S6.** Barplots showing the genotypic variability in sensitivity of E_{leaf} to ABA.

584 **Supplemental figure S7.** Sensitivity of leaf water potential (Ψ_{leaf}) to ABA fed to detached leaves for
585 10 offspring genotypes of the Syrah \times Grenache population and the parents.

586 **Supplemental method S8.** Details of the equation and parameters values used in the modelling of
587 midday leaf water potential response to soil drying in iso and aniso-hydric genotypes as a function of
588 K_{leaf} sensitivity to ABA.

589 **Supplemental figure S9.** Common relationship for isohydric and anisohydric genotypes used for
590 modelling the concentration of ABA in the xylem sap at predawn.

591 **Supplemental figure S10.** Relationship used for modelling stomatal conductance as a function of
592 concentration of ABA in guard cells apoplast and water potential of guard cells.

593

594

595 Supplemental Data Legends

596 **Supplemental figure S1.** Response of stomatal conductance (g_s) to ABA for the parental cultivars
597 Syrah and Grenache, measured on detached leaves fed with artificial sap containing variable ABA
598 concentration in the “HPFM experiment”.

599 **Supplemental figure S2.** Example of raw plots from the HPFM experiments

600 **Supplemental figure S3.** Same data as in Figure 1 showing effect of exogenous ABA on hydraulic
601 conductance of detached leaves (K_{leaf}) from 10 offspring genotypes and the parents, except that data is
602 plotted in semi logarithmic coordinates to linearize the response (K_{leaf} as a function of $\ln([\text{ABA}])$ in
603 the feeding sap).

604 **Supplemental figure S4.** Response of transpiration rate (E_{leaf}) to exogenous ABA measured on
605 detached leaves for the 10 grapevines offspring genotypes and the parents.

606 **Supplemental figure S5.** Transpiration rate of detached leaves (E_{leaf}) as a function of $\ln([\text{ABA}])$ in the
607 feeding sap; same data as in Figure S4 but plotted in semi logarithmic coordinates to linearize the
608 response (E_{leaf} as a function of $\ln([\text{ABA}])$ in the feeding sap).

609 **Supplemental figure S6.** Barplots showing the genotypic variability in sensitivity of E_{leaf} to ABA.

610 **Supplemental figure S7.** Sensitivity of leaf water potential (Ψ_{leaf}) to ABA fed to detached leaves for
611 10 offspring genotypes of the Syrah \times Grenache population and the parents.

612 **Supplemental method S8.** Details of the equation and parameters values used in the modelling of
613 midday leaf water potential response to soil drying in iso and aniso-hydric genotypes as a function of
614 K_{leaf} sensitivity to ABA.

615 **Supplemental figure S9.** Common relationship for isohydric and anisohydric genotypes used for
616 modelling the concentration of ABA in the xylem sap at predawn ($[\text{ABA}]_{\text{xyl_pd}}$).

617 **Supplemental figure S10.** Relationship used for modelling stomatal conductance (g_s) as a function of
618 concentration of ABA in guard cells apoplast ($[\text{ABA}]_{\text{gc}}$) and water potential of guard cells (Ψ_{gc}),
619 common for isohydric and anisohydric genotypes.

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622 in plant preparation, high-throughput experiments and measurements on detached leaves. We are also
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624 running ABA analysis with the LCMS.

625 In memory of our dear colleague and friend Eric Lebon.

626 **Figures Legends**

627 **Fig. 1. Effect of ABA on hydraulic conductance of detached leaves (K_{leaf}), laminas (K_{lamina}) and**
628 **petioles (K_{petiole}) sampled on cultivars Grenache and Syrah. Hydraulic conductance expressed as**
629 **a function of $\ln([\text{ABA}])$ in the feeding sap.** (A, B) Excised leaves from potted plants were perfused
630 for one hour in artificial sap containing no ABA, or ABA at concentration 2, 4, 8, 16 or 32 mmol m^{-3}
631 (+)-ABA prior to the determination of K_{leaf} with the Evaporative Flux Method (EFM). (C, D) Shoots
632 were cut from a vineyard and rehydrated overnight prior to leaf excision, perfusion in artificial sap
633 containing no ABA or ABA at concentration 2, 4, 8, 16 or 32 mmol m^{-3} (+)-ABA, and measurement of
634 K_{leaf} with the High Pressure Flux Meter (HPFM). (E, F) The lamina was then removed by excising at
635 the petiole junction, and hydraulic conductance of the petiole (K_{petiole}) was determined with the HPFM.
636 (G, H) Hydraulic conductance of the lamina (K_{lamina}) was derived from K_{leaf} and K_{petiole} according to

637 the Ohm's law analogy considering that petiole and lamina transport water in series: $1/K_{\text{leaf}} = 1/K_{\text{petiole}}$
638 $+ 1/K_{\text{lamina}}$.

639 **Fig. 2. Characterisation of (an)isohydric behaviours for a selection of ten offspring genotypes as**
640 **compared to the whole progeny and the parents Syrah and Grenache.** Distribution of the
641 genotypic means recorded in the whole Syrah \times Grenache population for: (A) leaf water potential
642 measured in the daytime under water deficit conditions ($\Psi_{M \text{ WD}}$), and (B) reduction in leaf water
643 potential in the daytime under water deficit as compared to well-watered conditions ($\Delta\Psi_M$), as an
644 indicator of the (an)isohydric behaviour. In (A) and (B), data are genotypic means calculated for the
645 experiment conducted in 2012 presented in Coupel-Ledru et al. (2014) for $n=188$ genotypes. Values
646 for the panel of 10 offspring genotypes and for the parental genotypes Syrah and Grenache (notified as
647 'Syr' and 'Gre') are indicated with black arrows.

648 **Fig. 3. Concentration of ABA in the xylem sap ($[ABA]_{\text{xylem sap, midday}}$) of 10 offspring genotypes**
649 **selected in the Syrah \times Grenache population and the parents, and relationship with the**
650 **(an)isohydric behaviour.** (A) ABA concentration in the xylem sap expressed at midday under
651 controlled transpiring conditions from leaves of intact plants submitted to a moderate soil water
652 deficit. ABA was quantified by LCMS. Means \pm standard error for $n=2$ leaves sampled on 2 different
653 plants per genotype. Genotypes are ordered according to their (an)isohydric behaviour as estimated by
654 the difference in leaf water potential ($\Delta\Psi_M$) between water deficit and well-watered conditions
655 measured under controlled transpiring conditions (like in Fig. 2 B). Genotypes without common letters
656 above their respective bars have significantly different $[ABA]_{\text{xylem sap, midday}}$ ($p<0.05$). (B) Correlation
657 between ABA concentration in the xylem sap and (an)isohydry level ($\Delta\Psi_M$). The correlation is not
658 significant ($p=1$).

659 **Fig. 4. Sensitivity of leaf hydraulic conductance (K_{leaf}) to ABA for 10 offspring genotypes of the**
660 **Syrah \times Grenache population and the parents.** K_{leaf} was measured on detached leaves fed with
661 artificial sap containing variable ABA concentration. Each panel (A-L) represents a genotype, and
662 genotypes are ordered from the highest (A) to the lowest (L) sensitivity of K_{leaf} to ABA. K_{leaf} was
663 measured according to the Evaporative Flux Method. Mean \pm standard error for $n=5$ leaves taken from
664 2 to 3 plants measured at each concentration for each genotype. Fitting of linear regression models
665 was performed on semi-ln transformed data (Fig. S3) and are represented here by the lines in the non-
666 transformed coordinates.

667 **Fig. 5. Variability in maximum leaf hydraulic conductance ($K_{\text{leaf max}}$), in sensitivity of leaf**
668 **hydraulic conductance (K_{leaf}) to ABA and its relationships with the (an)isohydric behaviour**
669 **estimated at the whole plant level for 10 offspring genotypes of the Syrah \times Grenache population**
670 **and the parents.** (A) Variability in maximum value of K_{leaf} without ABA treatment (means and SEs
671 of values measured for $n=5$ leaves per genotype). Comparison using Kruskal-Wallis test revealed a

672 significant effect of the genotype in $K_{\text{leaf max}}$ ($p < 0.05$). Genotypes without common letters above their
673 respective bars have significantly different $K_{\text{leaf max}}$. (B) Variability in sensitivity of K_{leaf} to ABA,
674 calculated as the opposite of the slope of the linearized relationship between K_{leaf} in $\text{mmol m}^{-2} \text{s}^{-1}$
675 MPa^{-1} and $\ln([\text{ABA}]$ in the feeding sap expressed in mmol m^{-3}), as presented in supplemental figure
676 S3. A higher sensitivity represents a stronger downregulation by ABA. Bars indicate the confidence
677 interval. Genotypes without common letters above their respective bar have significantly different K_{leaf}
678 sensitivity to ABA. (C) Relationship between K_{leaf} sensitivity to ABA and $K_{\text{leaf max}}$. (D) Relationship
679 between K_{leaf} sensitivity to ABA and the (an)isohydric behaviour estimated at the whole plant level as
680 the difference in leaf water potential measured in the daytime between water deficit and well-watered
681 conditions ($\Delta\Psi_M$) as in Fig. 2 B. In (C) and (D) Pearson's determination coefficient is indicated with
682 its significance level: $**p < 0.001$.

683 **Fig. 6. Correlation between % reduction in leaf hydraulic conductance (% reduction K_{leaf}) and**
684 **% reduction in transpiration rate (% reduction E_{leaf}) predicted from assays on detached leaves**
685 **(A) or (B) measured directly on intact plants (% reduction E_{plant}) for the 10 offspring genotypes**
686 **selected in the Syrah \times Grenache population and the parents.** Predicted changes in K_{leaf} and E_{leaf}
687 between well-watered and water deficit conditions were calculated assuming maximum K_{leaf} in well-
688 watered plants and combining ABA concentration measured in xylem sap of intact plants for each
689 genotype under water deficit (Fig. 3) with response to ABA assayed on detached leaves (Fig. 5).
690 Difference between well-watered and water deficit condition are expressed as a fraction of values
691 observed under well-watered or no ABA conditions. E_{plant} was measured on leaves of 3 intact plants
692 under well-watered conditions and on leaves of 3 other intact plants under water deficit conditions.
693 Pearson's determination coefficients are indicated with their significance level: $**p < 0.001$, $*p < 0.1$.
694 The dashed grey line corresponds to the 1:1 relationship.

695 **Fig. 7. Modelling confirms the influence of differential downregulation of K_{leaf} by ABA on the**
696 **overall (an)isohydric behaviour of the plant.** Two simulations were performed with all parameters
697 maintained identical in both cases apart from K_{leaf} sensitivity to ABA and K_{leaf} maximum value without
698 ABA (A and D). Assumptions made to simulate the effects of a progressive soil drying, characterized
699 by the drop in the predawn leaf water potential (Ψ_{pd} in B and E), are described in the Material and
700 Method section. Left part: high sensitivity of K_{leaf} to ABA (A) drives a drop in the guard cell water
701 potential Ψ_{gc} (B) resulting in a decrease in stomatal conductance (C) and transpiration rate which, in
702 turn, reduces the drop in the leaf water potential Ψ_{leaf} (B) and confers a more isohydric behaviour.
703 Right part: lower sensitivity of K_{leaf} to ABA (D) leads to a higher stomatal conductance (F) as
704 compared to (C), not preventing the drop in the overall leaf water potential (E), typical of a more
705 anisohydric behaviour.

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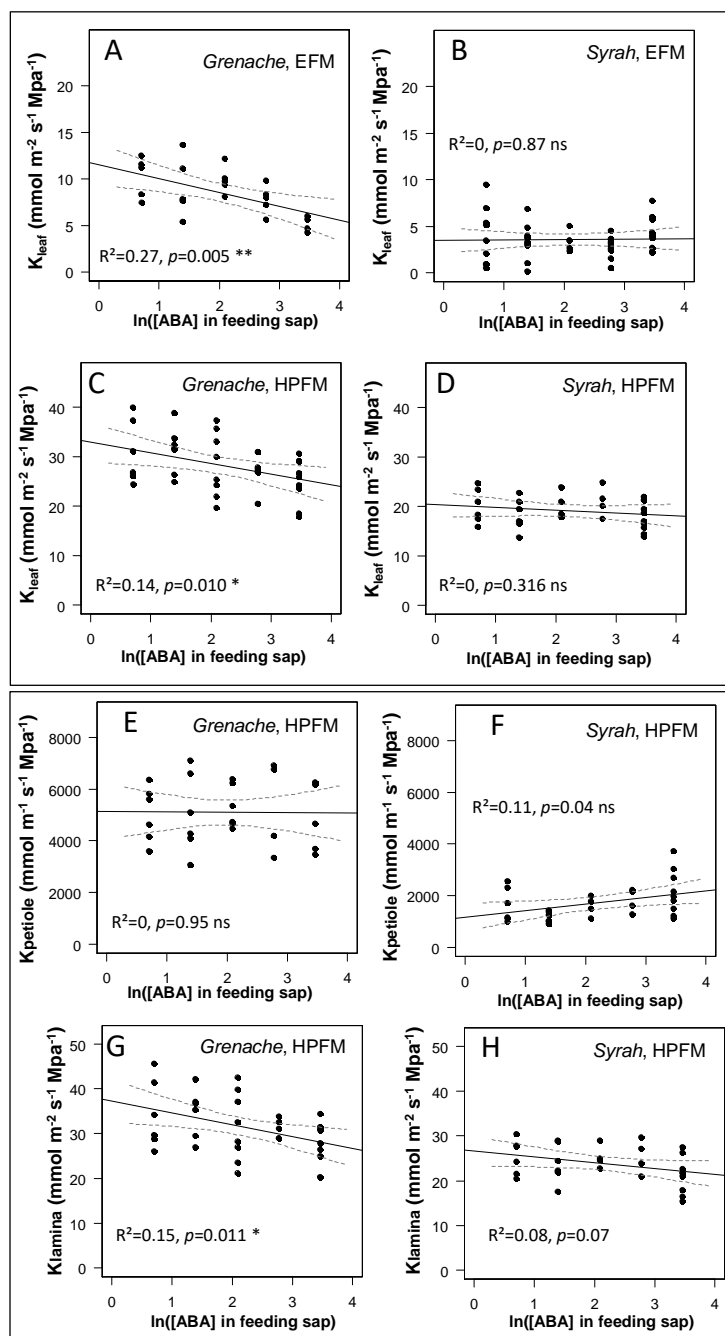


Fig. 1. Effect of ABA on hydraulic conductance of detached leaves (K_{leaf}), lamina (K_{lamina}) and petioles (K_{petiole}) sampled on cultivars Grenache and Syrah. Hydraulic conductance expressed as a function of $\ln([\text{ABA}] \text{ in the feeding sap})$. (A, B) Excised leaves from potted plants were perfused for one hour in artificial sap containing no ABA, or ABA at concentration 2, 4, 8, 16 or 32 mmol m^{-3} (+)-ABA prior to the determination of K_{leaf} with the Evaporative Flux Method (EFM). (C, D) Shoots were cut from a vineyard and rehydrated overnight prior to leaf excision, perfusion in artificial sap containing no ABA or ABA at concentration 2, 4, 8, 16 or 32 mmol m^{-3} (+)-ABA, and measurement of K_{leaf} with the High Pressure Flow Meter (HPFM). (E, F) The lamina was then removed by excising at the petiole junction, and hydraulic conductance of the petiole (K_{petiole}) was determined with the HPFM. (G, H) Hydraulic conductance of the lamina (K_{lamina}) was derived from K_{leaf} and K_{petiole} according to the Ohm's law analogy considering that petiole and lamina transport water in series: $1/K_{\text{leaf}} = 1/K_{\text{petiole}} + 1/K_{\text{lamina}}$.

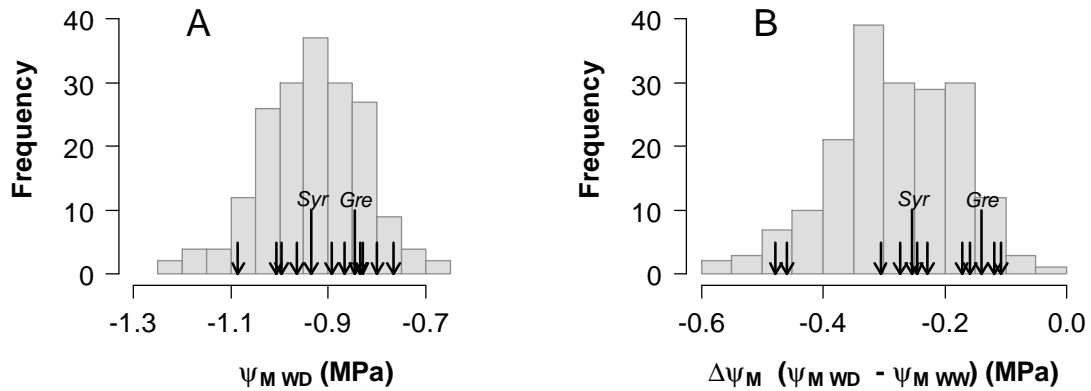


Fig. 2. Characterisation of (an)isohydric behaviours for a selection of ten offspring genotypes as compared to the whole progeny and the parents Syrah and Grenache. Distribution of the genotypic means recorded in the whole Syrah \times Grenache population for: (A) leaf water potential measured in the daytime under water deficit conditions ($\Psi_{M\text{ WD}}$), and (B) reduction in leaf water potential in the daytime under water deficit as compared to well-watered conditions ($\Delta\Psi_M$), as an indicator of the (an)isohydric behaviour. In (A) and (B), data are genotypic means calculated for the experiment conducted in 2012 presented in Coupel-Ledru et al. (2014) for $n=188$ genotypes. Values for the panel of 10 offspring genotypes and for the parental genotypes Syrah and Grenache (notified as 'Syr' and 'Gre') are indicated with black arrows.

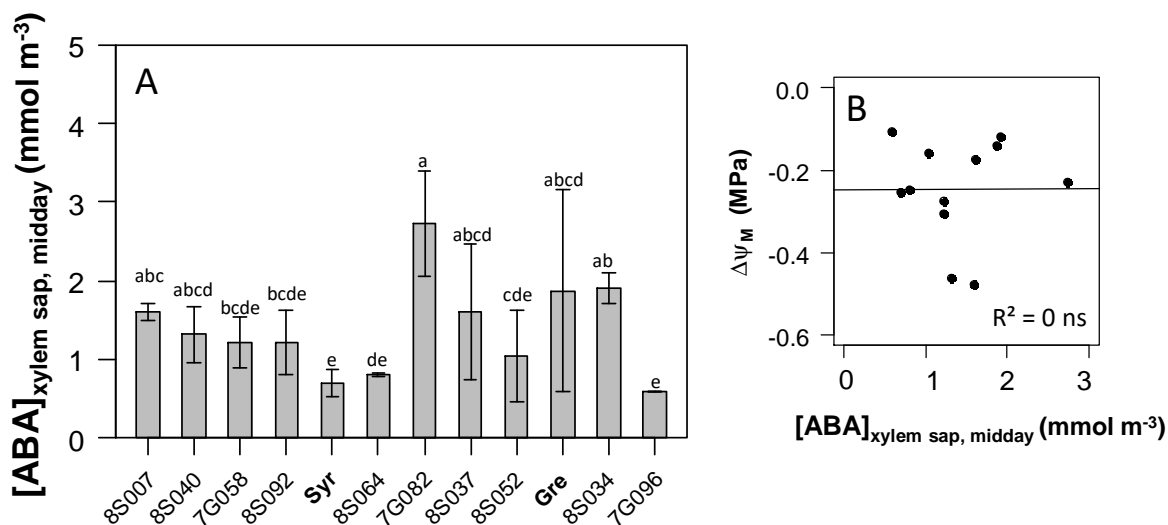


Fig. 3. Concentration of ABA in the xylem sap ([ABA]_{xylem sap, midday}) of 10 offspring genotypes selected in the Syrah × Grenache population and the parents, and relationship with the (an)isohydric behaviour. (A) ABA concentration in the xylem sap expressed at midday under controlled transpiring conditions from leaves of intact plants submitted to a moderate soil water deficit. ABA was quantified by LCMS. Means ± standard error for $n=2$ leaves sampled on 2 different plants per genotype. Genotypes are ordered according to their (an)isohydric behaviour as estimated by the difference in leaf water potential ($\Delta\Psi_M$) between water deficit and well-watered conditions measured under controlled transpiring conditions (like in Fig. 2 B). Genotypes without common letters above their respective bars have significantly different [ABA]_{xylem sap, midday} ($p<0.05$). (B) Correlation between ABA concentration in the xylem sap and (an)isohydry level ($\Delta\Psi_M$). The correlation is not significant ($p=1$).

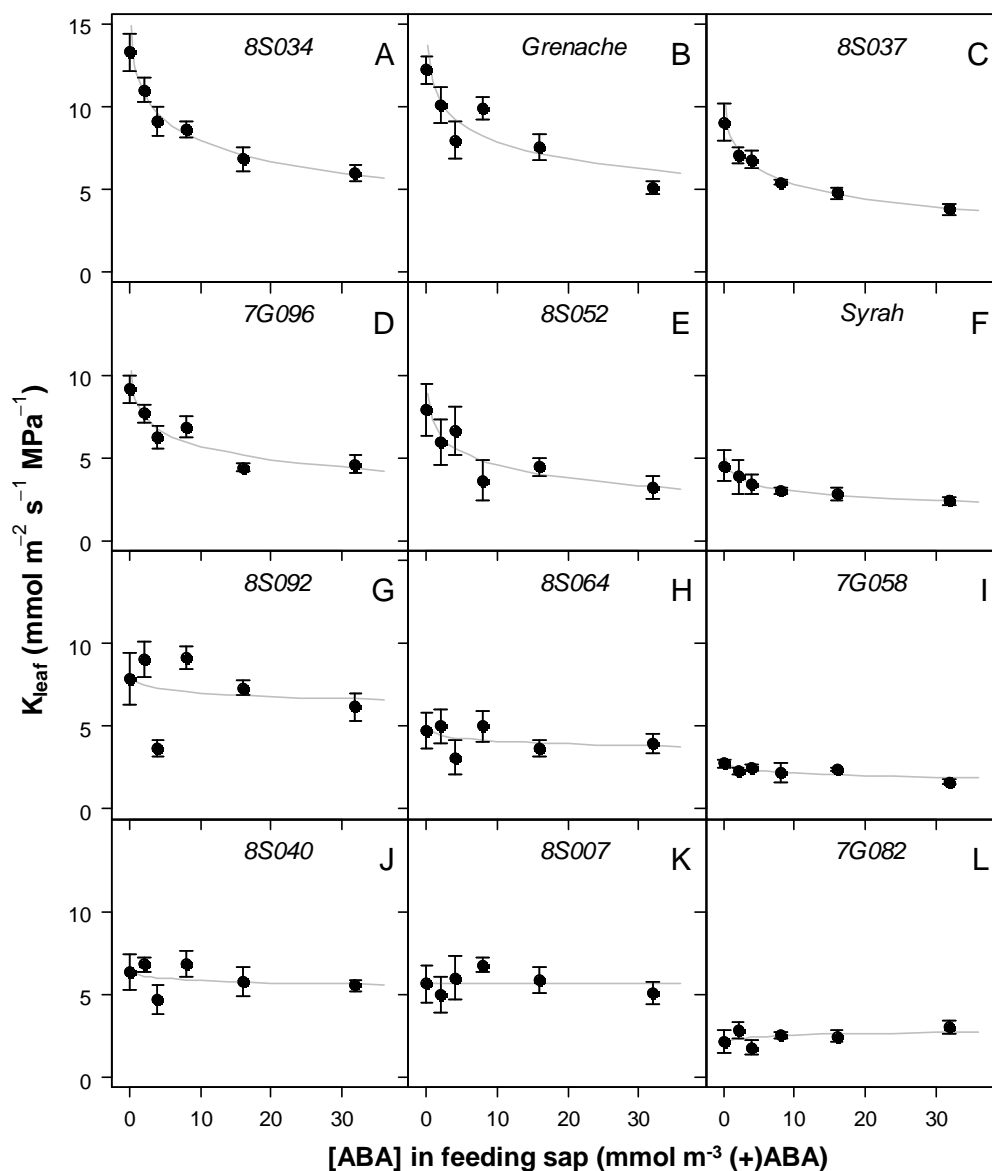


Fig. 4. Sensitivity of leaf hydraulic conductance (K_{leaf}) to ABA for 10 offspring genotypes of the Syrah \times Grenache population and the parents. K_{leaf} was measured on detached leaves fed with artificial sap containing variable ABA concentration. Each panel (A-L) represents a genotype, and genotypes are ordered from the highest (A) to the lowest (L) sensitivity of K_{leaf} to ABA. K_{leaf} was measured according to the Evaporative Flux Method. Mean \pm standard error for $n=5$ leaves taken from 2 to 3 plants measured at each concentration for each genotype. Fitting of linear regression models was performed on semi-ln transformed data (Fig. S3) and are represented here by the lines in the non-transformed coordinates.

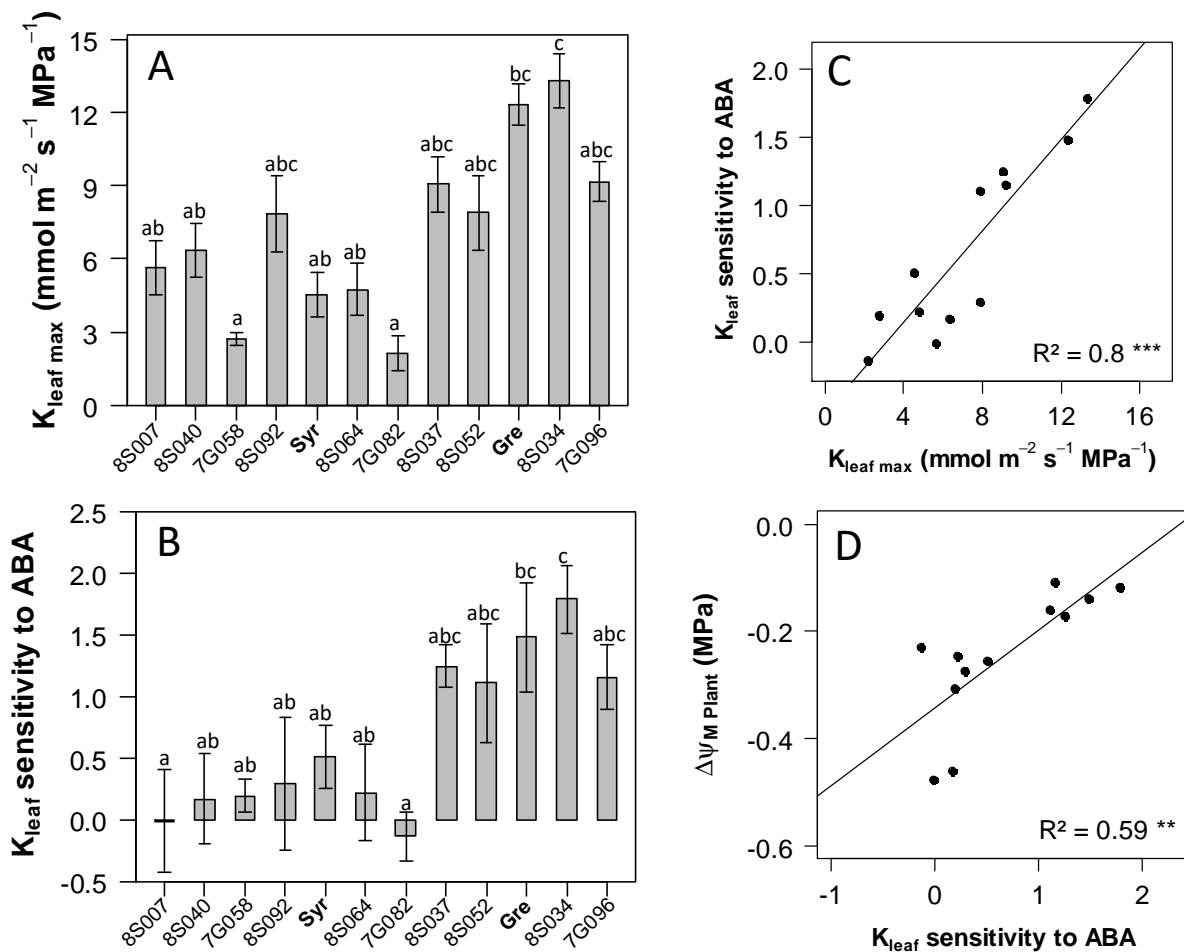


Fig. 5. Variability in maximum leaf hydraulic conductance ($K_{\text{leaf max}}$), in sensitivity of leaf hydraulic conductance (K_{leaf}) to ABA and its relationships with the (an)isohydric behaviour estimated at the whole plant level for 10 offspring genotypes of the Syrah \times Grenache population and the parents. (A) Variability in maximum value of K_{leaf} without ABA treatment (means and SEs of values measured for $n=5$ leaves per genotype). Comparison using Kruskal-Wallis test revealed a significant effect of the genotype in $K_{\text{leaf max}}$ ($p<0.05$). Genotypes without common letters above their respective bars have significantly different $K_{\text{leaf max}}$. (B) Variability in sensitivity of K_{leaf} to ABA, calculated as the opposite of the slope of the linearized relationship between K_{leaf} in $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ and $\ln([\text{ABA}]$ in the feeding sap expressed in mmol m^{-3}), as presented in supplemental figure S3. A higher sensitivity represents a stronger downregulation by ABA. Bars indicate the confidence interval. Genotypes without common letters above their respective bar have significantly different K_{leaf} sensitivity to ABA. (C) Relationship between K_{leaf} sensitivity to ABA and $K_{\text{leaf max}}$. (D) Relationship between K_{leaf} sensitivity to ABA and the (an)isohydric behaviour estimated at the whole plant level as the difference in leaf water potential measured in the daytime between water deficit and well-watered conditions ($\Delta\Psi_M$) as in Fig. 2B. In (C) and (D) Pearson's determination coefficient is indicated with its significance level: ** $p<0.001$.

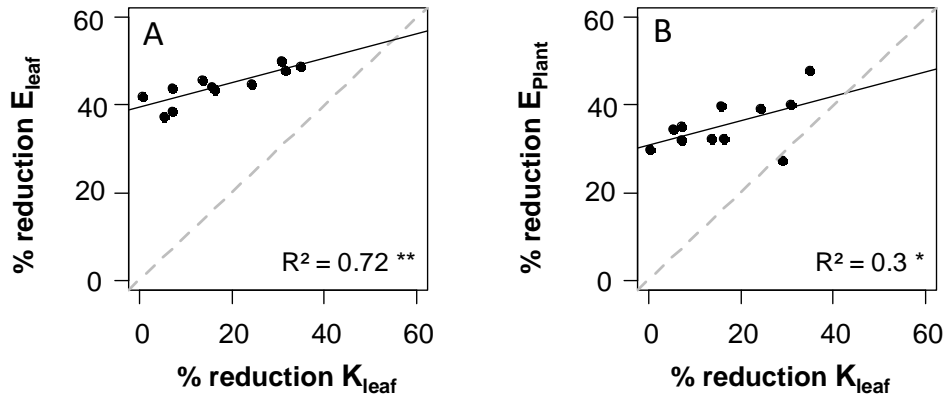


Fig. 6. Correlation between % reduction in leaf hydraulic conductance (% reduction K_{leaf}) and % reduction in transpiration rate (% reduction E_{leaf}) predicted from assays on detached leaves (A) or (B) measured directly on intact plants (% reduction E_{Plant}) for the 10 offspring genotypes selected in the Syrah \times Grenache population and the parents. Predicted changes in K_{leaf} and E_{leaf} between well-watered and water deficit conditions were calculated assuming maximum K_{leaf} in well-watered plants and combining ABA concentration measured in xylem sap of intact plants for each genotype under water deficit (Fig. 3) with response to ABA assayed on detached leaves (Fig. 5). Difference between well-watered and water deficit condition are expressed as a fraction of values observed under well-watered or no ABA conditions. E_{Plant} was measured on leaves of 3 intact plants under well-watered conditions and on leaves of 3 other intact plants under water deficit conditions. Pearson's determination coefficients are indicated with their significance level: ** $p < 0.001$, * $p < 0.1$. The dashed grey line corresponds to the 1:1 relationship.

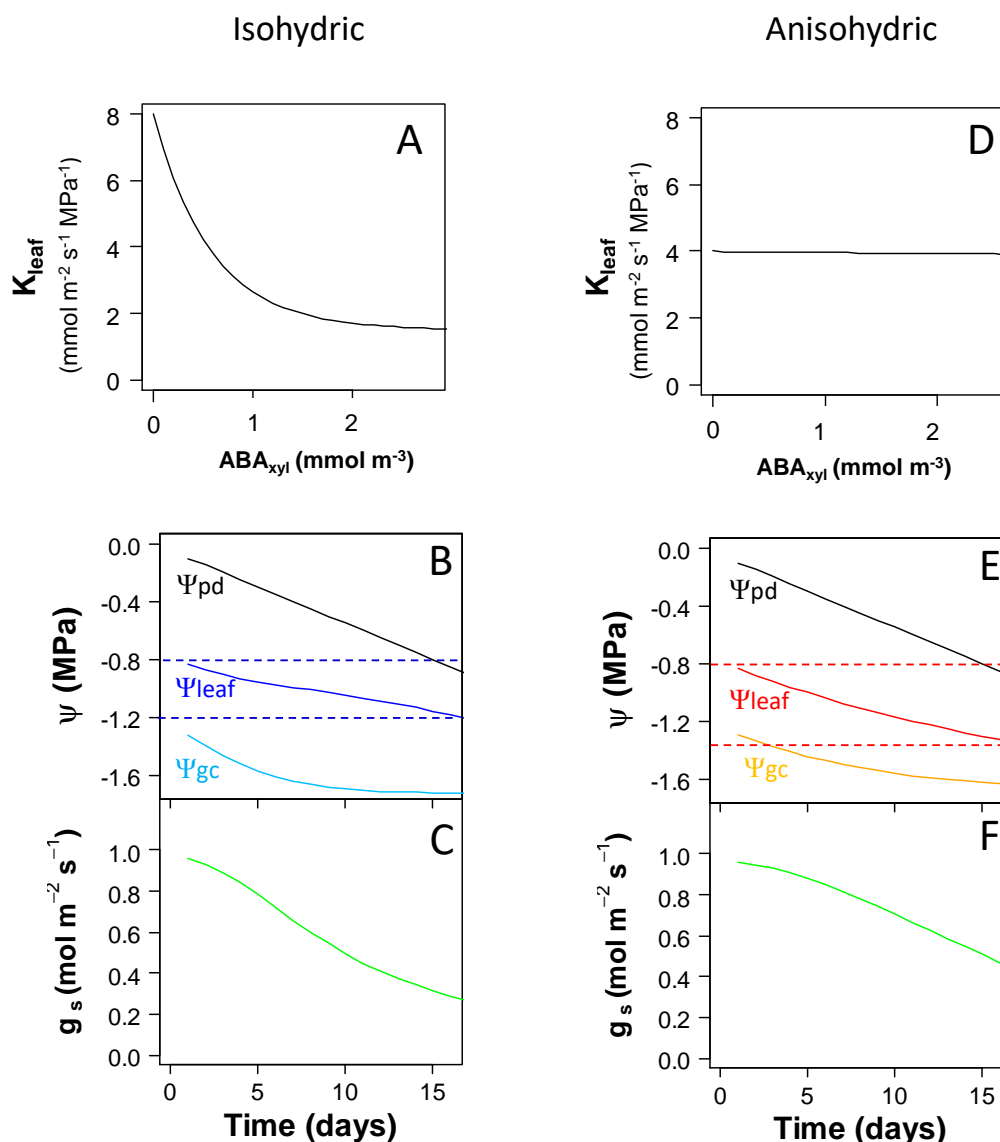


Fig. 7. Modelling confirms the influence of differential downregulation of K_{leaf} by ABA on the overall (an)isohydric behaviour of the plant. Two simulations were performed with all parameters maintained identical in both cases apart from K_{leaf} sensitivity to ABA and K_{leaf} maximum value without ABA (A and D). Assumptions made to simulate the effects of a progressive soil drying, characterized by the drop in the predawn leaf water potential (Ψ_{pd} in B and E), are described in the Material and Method section. Left part: high sensitivity of K_{leaf} to ABA (A) drives a drop in the guard cell water potential Ψ_{gc} (B) resulting in a decrease in stomatal conductance (C) and transpiration rate which, in turn, reduces the drop in the leaf water potential Ψ_{leaf} (B) and confers a more isohydric behaviour. Right part: lower sensitivity of K_{leaf} to ABA (D) leads to a higher stomatal conductance (F) as compared to (C), not preventing the drop in the overall leaf water potential (E), typical of a more anisohydric behaviour.

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