Genetic variation in a grapevine progeny (Vitis vinifera L. cvs GrenachexSyrah) reveals inconsistencies between maintenance of daytime leaf water potential and response of transpiration rate under drought

Aude Coupel-Ledru, Eric Lebon, Angélique Christophe, Agnes Doligez, Llorenç Cabrera Bosquet, Philippe Péchier, Philippe Hamard, Patrice This, Thierry Simonneau

To cite this version:
Aude Coupel-Ledru, Eric Lebon, Angélique Christophe, Agnes Doligez, Llorenç Cabrera Bosquet, et al.. Genetic variation in a grapevine progeny (Vitis vinifera L. cvs GrenachexSyrah) reveals inconsistencies between maintenance of daytime leaf water potential and response of transpiration rate under drought. Journal of Experimental Botany, Oxford University Press (OUP), 2014, 65 (21), 14 p. 10.1093/jxb/eru228. hal-01054469

HAL Id: hal-01054469
https://hal.archives-ouvertes.fr/hal-01054469
Submitted on 21 Mar 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Genetic variation in a grapevine progeny (Vitis vinifera L. cvs Grenache×Syrah) reveals inconsistencies between maintenance of daytime leaf water potential and response of transpiration rate under drought

Aude Coupel-Ledru1, Éric Lebon1, Angélique Christophe1, Agnès Doligez2, Llorenç Cabrera-Bosquet1, Philippe Péchier1, Philippe Hamard1, Patrice This2 and Thierry Simonneau1,*

1 INRA Laboratoire d’Ecophysiologie des Plantes sous Stress Environnementaux, Place Viala, F-34060 Montpellier Cedex 1, France
2 INRA UMR AGAP, Place Viala, F-34060 Montpellier Cedex 1, France

* To whom correspondence should be addressed. E-mail: simonnea@supagro.inra.fr

Received 12 January 2014; Revised 14 April 2014; Accepted 22 April 2014

Abstract

In the face of water stress, plants evolved with different abilities to limit the decrease in leaf water potential, notably in the daytime (Ψ_M). So-called isohydric species efficiently maintain high Ψ_M, whereas anisohydric species cannot prevent Ψ_M from dropping as soil water deficit develops. The genetic and physiological origins of these differences in (an)isohydric behaviours remain to be clarified. This is of particular interest within species such as Vitis vinifera L. where continuous variation in the level of isohydry has been observed among cultivars. With this objective, a 2 year experiment was conducted on the pseudo-F_1 progeny from a cross between the two widespread cultivars Syrah and Grenache using a phenotyping platform coupled to a controlled-environment chamber. Potted plants of all the progeny were analysed for Ψ_M, transpiration rate, and soil-to-leaf hydraulic conductance, under both well-watered and water deficit conditions. A high genetic variability was found for all the above traits. Four quantitative trait loci (QTLs) were detected for Ψ_M under water deficit conditions, and 28 other QTLs were detected for the different traits in either condition. Genetic variation in Ψ_M maintenance under water deficit weakly correlated with drought-induced reduction in transpiration rate in the progeny, and QTLs for both traits did not completely co-localize. This indicates that genetic variation in the control of Ψ_M under water deficit was not due simply to variation in transpiration sensitivity to soil drying. Possible origins of the diversity in (an)isohydric behaviours in grapevine are discussed on the basis of concurrent variations in soil-to-leaf hydraulic conductance and stomatal control of transpiration.

Key words: Anisohydric, drought, high-throughput phenotyping, hydraulic conductance, isohydric, leaf water potential, QTL, transpiration rate, Vitis vinifera L.

Introduction

Soil water deficit (WD) represents the main environmental constraint for growth in grapevine (Vitis vinifera L.) and grape production under Mediterranean conditions (Chaves et al., 2010). When combined with high evaporative demand, soil drying may result in a dramatic decrease in water potential in plant tissues. This can lead to catastrophic effects such as cavitation in the xylem vessels (Zufferey et al., 2011), cessation of water transport, stomatal closure, and carbon starvation, which threaten plant survival (McDowell et al., 2008). Plants have evolved various adaptive processes limiting a dramatic decrease in leaf water potential in the daytime (Ψ_M) under soil drought. As a result, contrasting controls of Ψ_M have been observed across species when submitted to similar soil WD conditions (Tardieu and Simonneau, 1998).
So-called isohydric species, such as maize, efficiently maintain high $\Psi_M$ when the soil dries, whereas anisohydric species, such as sunflower, cannot prevent $\Psi_M$ from dropping (Tardieu et al., 1996). In several species including grapevine (Prieto et al., 2010), genetically variable efficiency of $\Psi_M$ maintenance has been observed. Two widespread cultivars of grapevine, namely Grenache and Syrah, have been consistently described with different responses to soil WD. Grenache was shown to be near-isohydric, compared with Syrah which exhibited more anisohydric behaviour (Schultz, 2003; Soar et al., 2006).

It has been proposed that the variation between isohydric and anisohydric behaviours mainly results from how stomatal pores at the leaf surface close under WD and control plant transpiration (Buckley, 2005). With regard to this, stomatal conductance to water vapour has been shown to decrease for milder soil WD in maize (isohydric) than in sunflower (anisohydric) (Tardieu and Simonneau, 1998). The higher stomatal sensitivity to WD in isohydric compared with anisohydric species could therefore be the cause of the more efficient maintenance of $\Psi_M$. The drought-induced hormone abscisic acid, which triggers stomatal closure, was suspected to be at the origin of this difference (Tardieu and Davies, 1992; Borel et al., 1997; Tardieu and Simonneau, 1998). However, the physiological basis of this proposal has never been elucidated. Additionally, it has been contested that (an)isohydric behaviour was stable for a given genotype but could vary seasonally (Franks et al., 2007; Chaves, 2010).

Plant hydraulic conductance (i.e. a plant’s capacity to supply soil water to the leaves) participates in determining $\Psi_M$ as it balances the impact of water losses. Variation in plant hydraulic conductance was therefore proposed to contribute, concurrently with stomatal regulation, to the control of $\Psi_M$ under adverse conditions (Franks et al., 2007; Pantin et al., 2013). Accordingly, $\Psi_M$ should remain high in those plants which could maintain high hydraulic conductance under soil drying. Conversely, vulnerability of the xylem pathway to embolism under drought most often causes a decrease in hydraulic conductance as observed in field-grown grapevine (Zufferey et al., 2011), thereby lowering $\Psi_M$ and resulting in anisohydric behaviour. This potential role of hydraulic conductance is supported by studies where different responses of hydraulic conductivity in roots (Vandeleur et al., 2009) or petioles (Schultz, 2003) between grapevine cultivars under water stress have been related to their contrasted behaviours in terms of being isohydric or anisohydric.

The present study aims at elucidating whether and how variation in (an)isohydric behaviour is genetically controlled. This was addressed using a quantitative trait locus (QTL) mapping population consisting of the pseudo-F$_1$ progeny obtained from a reciprocal cross between the two cultivars Syrah and Grenache. The range of genetic variation in $\Psi_M$ observed in the progeny under controlled transpiring conditions was quantified with either a well-irrigated regime or moderate soil WD in pots. The genetic architecture underlying variation in $\Psi_M$ within the progeny was then examined by looking for genomic regions associated with the control of this trait using a QTL approach. The transpiration rate and $\Psi_M$ were characterized in parallel in all the progeny, and the genetic architecture of transpiration response to WD was similarly dissected. The physiological bases of isohydric versus anisohydric behaviours were discussed by matching correlations between traits to co-localizations between underlying QTLs.

**Materials and methods**

**Plant material and treatments in the greenhouse**

The plant material consisted of the pseudo-F$_1$ progeny of 186 two-year-old genotypes obtained as the first generation from a reciprocal cross between the grapevine cultivars Syrah and Grenache (Adam-Blondon et al., 2004).

In February 2010, 20 clones of each offspring and the parents were grafted on 110 Richter rootstock (Vitis berlandieri × Vitis rupestris) and then cultivated outside with ferti-irrigation in 9 litre (0.19 m diameter, 0.4 m high) individual pots containing a 30:70 (v/v) mixture of a loamy soil and organic compost. Six clones of each offspring and the parents were selected as replicates during a first experiment in 2012 (1128 two-year-old plants). Five other replicates (clones) were studied in 2013 (940 three-year-old plants).

In each year of the experiment, potted plants were transferred for budburst to a first greenhouse and grown there for 1 month. They were individually weighed and irrigated twice a week so as to maintain soil water content as non-limiting for growth (1.5 g of water per g of dry soil; see Supplementary Fig. S1 available at JXB online). All inflorescences and branches were removed in order to ensure full growth of one leafy axis per plant.

The plants were then transferred into the PhenoArch phenotyping platform located in another greenhouse where water treatments were imposed. Briefly, PhenoArch is based on a LemnaTec automated system (LemnaTec, Wüerselen, Germany) and high-precision pump-watering stations (520U, Watson Marlow, Wilmington, MA, USA). For each genotype (186 offspring plus two parents), well-watered (WW) replicates (three clones in 2012 and two in 2013) were maintained at 1.5 g of water per g of dry substrate. A moderate soil WD was imposed on three other replicates (clones) per genotype in each year, by restricting irrigation until the SWC gradually reached 1.05 g g$^{-1}$, and this level was maintained thereafter by daily watering for 1–3 d pending hydraulic measurements. Target weights used for daily irrigation were calculated for each pot as the sum of the desired amount of water corresponding to target SWC and individual tares which were determined during pot filling (empty pot, dry soil) including individual plant biomasses which were re-estimated twice a week by image analysis (see below). The target SWC for WD treatment was reached on average 7 d after restricting irrigation (Fig. 1) which was not long enough to alter the plant leaf area of WD plants compared with WW plants (see below).

In 2012, mean air temperature and vapour pressure deficit (VPD) in the platform greenhouse were 21.4 °C and 1.38 kPa during the night and 25.6 °C and 2.16 kPa during the day, and the photosynthetic photon flux density (PPFD) during the light period averaged 290 μmol m$^{-2}$ s$^{-1}$. In 2013, cloudier conditions resulted in lower values, with mean air temperature and VPD of 18.8 °C and 1.01 kPa during the night and 22.4 °C and 2.16 kPa during the day, with an average PPFD of 272 μmol m$^{-2}$ s$^{-1}$ during the light period.

**Image acquisition and analysis**

Plant RGB (1280 × 960) images taken from two horizontal, orthogonal directions plus one top view were captured every 2 d for all plants during the night-time (3D Scanalyzer, LemnaTec, GmbH, Wüerselen, Germany). Images were analysed to separate plant
Maintenance of daytime leaf water potential and response of transpiration rate under drought. Journal of Experimental Botany, 65 (21), 6205-6218. DOI: 10.1093/jxb/eru228

Fig. 1. Evolution of soil water potential ($\Psi_{\text{soil}}$) for potted grapevine plantlets cultivated in the greenhouse under both well-watered (WW, black circles) and water deficit (WD, grey circles) treatments (means and SEs for two replicates of the 186 Syrah×Grenache offspring and the two parents studied in 2013). The dashed arrow shows the beginning of water restriction. The black and grey arrows show the day of measurement of hydraulic traits, when $\Psi_{\text{soil}}$ was stabilized under both scenarios.

Air temperature and relative humidity (RH) were measured every 30 s (HMP35A probe, Oy, Helsinki, Finland) and the temperature was set to an average of 27 °C during the day (20 °C during the night). VPD was controlled by manipulating RH and was maintained at 2 ± 0.2 kPa during the light period. Light was provided in the chamber by a bank of sodium lamps that maintained the PPFD at ~480 μmol m⁻² s⁻¹ at the level where leaves were sampled.

Before being placed in the chamber, pots were bagged to prevent evaporation from the soil. Each pot was then weighed with 0.1 g accuracy (Sartorious balance, IB 34 EDEP, Gottingen, Germany) at the beginning of the light period and after a minimum of 6 h under constant light, temperature, and VPD conditions. Weight losses over the 6 h period were used to calculate average daytime transpiration rates on a whole plant (Tr) and a leaf area basis (TrS=Tr/LA, hereafter called the specific transpiration rate). Weight losses and LA were corrected by the weight and surface area of the leaf that was sampled for water potential measurement.

For each plant, SWC (g water g⁻¹ dry soil) was calculated using the weight ($W_{\text{total}}$) measured just before entering the controlled-environment chamber, corrected by the estimated plant biomass ($B_{\text{plant}}$), as:

$$SWC = \frac{W_{\text{total}} - B_{\text{plant}} - W_{\text{pot}} - W_{\text{dry soil}}}{W_{\text{dry soil}}}$$

with $W_{\text{pot}}$ the weight of the empty pot and $W_{\text{dry soil}}$ the weight of dry soil determined when plants were potted (2250 g on average). Error in the SWC was reduced to <0.8% (errors were ±10.7 g for $B_{\text{plant}}$ and ±1 g for $W_{\text{dry soil}}$, $W_{\text{pot}}$, and $W_{\text{total}}$, resulting in <0.1% error for the denominator and <0.7% error for the numerator).

The soil water potential ($\Psi_{\text{soil}}$) was then calculated for each plant using a calibration curve (Van Genuchten et al., 1980) previously established between SWC and $\Psi_{\text{soil}}$ using pre-dawn leaf water potential determined under non-transpiring conditions on fully irrigated plants as a proxy for $\Psi_{\text{soil}}$ (Supplementary Fig. S3 at JXB online):

$$\Psi_{\text{soil}} = \frac{(SWC - SWC_{\text{sat}})}{SWC_{\text{sat}}} - \frac{1}{m}$$

with SWC_{sat}=2, n=1.468, and $m=0.258$. Using this calibration curve, soil water potential could be estimated from SWC with an RMSE of 0.06 MPa (Supplementary Fig. S3 at JXB online). Pre-dawn leaf water potential was chosen as a proxy for $\Psi_{\text{soil}}$ since it incorporates the influences of root distribution and distributed soil water potential in the pot on how the plant senses soil water availability. Further, calculation of water potential differences between transpiring leaves and soil (see below) was based on the same measurement technique.

The leaf water potential under transpiring conditions, as indicative of minimal daytime values (denoted $\Psi_M$), was measured on the plants in the controlled-environment chamber between 3 h and 4 h after lights were switched on, with up to six Scholander pressure chambers (Soil Moisture Equipment Corp., Santa Barbara, CA, USA) which were cross-calibrated using a distributed, pressurized nitrogen source. Measurements were performed on fully expanded, well-irradiated leaves, generally on the eighth phytomer from the apex. The drop in water potential between soil and transpiring leaves was calculated as:

$$\Delta\Psi = \Psi_{\text{soil}} - \Psi_M$$

Whole-plant (K) and specific (KS) soil-to-leaf hydraulic conductance were then deduced using the conventional, evaporative flux method (Tsuda and Tyree, 2000) for the whole path from soil to transpiring leaves as:

$$K = Tr / \Delta\Psi$$

Experimental design

The experimental design consisted of parallel blocks in the phytotyping platform, and one clone of each genotype was randomly positioned within each block. Plant development was followed twice a week by counting the number of unfolded leaves. To eliminate uncontrolled plant size effects on water relations (Ambroise et al., 2009), hydraulic traits were measured at a similar mean developmental stage for the total progeny except for two replicates of WW in 2013; and LA=0.238 m² for WD plants in 2012; LA=0.242 m² for WD in 2013).

Measurements of water relations in a controlled-environment chamber

Water potential and transpiration rate were measured under controlled transpiring conditions while plants were taken off the platform and placed in a controlled-environment chamber. Plant density in the chamber was reduced by one-third in 2013 compared with 2012 in order to facilitate sampling of exposed leaves. Plants were first submitted to a dark period with similar timing to that in the greenhouse (for ~12 h). Lights were then switched on for a minimum of 3 h before water potential measurements were performed.
and

\[ KS = \Delta Tr / \Delta \Psi \]

The stability of the transpiration rate over the 6h light period was verified in a preliminary experiment to ensure that Tr could be associated with the leaf water potential measured at any time during this period (Supplementary Fig. S4 at JXB online).

Statistical analyses

All statistical analyses were performed with R packages (R Development Core Team, 2012). Natural logarithm transformation was applied when data distribution deviated from normality (Shapiro–Wilk test; Royston, 1995). Single effects of year, water scenario, and genotype were first tested on all traits. Effects of water scenario and interaction with genotype were then tested within each year (2012 and 2013) by using the following analysis of variance (ANOVA) model: 

\[ P_i = \mu + G_i + S_j + G_i S_j + e_{ij} \]

where \( P_i \) was the phenotypic value of genotype \( i \) in scenario \( j, \) \( \mu \) the overall mean, \( G_i \) the effect of genotype \( i, \) \( S_j \) the effect of scenario \( j, \) and \( e_{ij} \) the residual error effect. The effect of year and interaction with genotype were tested within each scenario (WW and WD) using the same model, with \( S_j \) replaced by the effect of year \( Y_j. \)

Further analyses were carried out on separate data sets (e.g. WD or WW, 2012 or 2013) to test for undesired effects (spatial position in the platform, date of measurement, and operator for leaf water potential measurement) by using one-way ANOVA models such as: 

\[ P_i = \mu + X_i + e_i \]

with \( X_i \) an undesired effect, and \( e_i \) the corresponding residual.

For each trait, the Best Linear Unbiased Predictors (BLUPs) of genetic values were then estimated for use in QTL detection. Models selected were those with the lowest Bayesian Information Criterion, among several mixed models (Supplementary Table S1 at JXB online). The tested models always included a random genetic effect, completed or not by fixed effects and interactions reported as significant in previous ANOVAs for each data set (water scenario×year, multiscenarios, multiyears, all years and scenarios). Pearson’s correlation coefficients between years or traits were calculated on BLUPs of the genetic value.

Results

Mean effects of water treatments

All plants which were characterized in a controlled-environment chamber reached the desired soil water potential corresponding to either WW or WD treatment with high reproducibility between plants (the SE on \( \Psi_{soil} \) was <0.0014 MPa and 0.014 MPa for WW and WD plants, respectively) and years (mean \( \Psi_{soil} \) for all WD plants averaged –0.154 ± 0.041 MPa in 2012 and –0.151 ± 0.005 MPa in 2013).

WD treatment resulted in a highly significant decrease of all studied traits, with highly repeatable mean effects on plants between years (Table 1, Fig. 2). Mean \( \Psi_{soil} \) for all measured plants was substantially reduced from –0.66 MPa under WW conditions to –0.94 MPa under WD treatment (Table 1, Fig. 2). Specific transpiration rate (TrS) and specific hydraulic conductance (KS) were slightly but significantly higher in 2013 compared with 2012. In spite of this difference, reduction induced by WD compared with WW conditions was of similar magnitude between years for mean KS, decreasing down to 43%, and mean TrS, which decreased down to 55% (Table 1, Fig. 2).

Genetic variability within the progeny and comparison with parents

Mean genotypic values (BLUPs) were calculated using three replicates of each genotype submitted to the same treatment in each year (but only two for WW in 2013). Correlations between years were significant for each trait (as exemplified in Fig. 3 for WD) although with relatively low \( R^2 \) values (0.06–0.3), suggesting an interaction between genotypes and years.

Parents were ranked in agreement with their previously reported behaviours under WD, with higher \( \Psi_{soil} \) in the near-isohydric Grenache compared with the near-anisohydric Syrah (Fig. 4A). However, the difference between parents was rather weak compared with the range of variation observed among offspring.
AN OVA revealed highly significant effects of the genotype on $\Psi_M$ under WD treatment but not under WW treatment, where mean genotypic values for $\Psi_M$ were maintained between –0.78 MPa and –0.6 MPa (not shown). Segregation of $\Psi_M$ under WD treatment within the progeny was therefore indicative of differences in (an)isohydric behaviours. Mean genotypic values of $\Psi_M$ under WD ranged from –0.79 MPa in 2012 (–0.78 MPa in 2013), indicative of the most isohydratic behaviour, to –1.12 MPa in 2012 (–1.07 MPa in 2013), indicative of the most anisohydric response (Fig. 4A). Genetic variability in (an)isohydric behaviours could also be appreciated through the range of variation in the reduction of $\Psi_M$ induced by WD (Red$_{\Psi_M}$), going from –0.17 MPa for the most isohydratic offspring to –0.40 MPa for the most anisohydratic one (Fig. 4D). However, Red$_{\Psi_M}$ was obtained as the mean of only two values (one in each year; see the Materials and methods), while mean genotypic $\Psi_M$ was calculated using three replicates in each year and was therefore preferred for assessing (an)isohydric behaviours.

A large and transgressive variability among offspring was also observed for both specific transpiration rate and specific hydraulic conductance (Fig. 4B, C). Mean, genotypic values under WD conditions ranged from 0.24 mmol m$^{-2}$ s$^{-1}$ to 0.76 mmol m$^{-2}$ s$^{-1}$ for Tr$_S$ and from 0.28 mmol m$^{-2}$ s$^{-1}$ MPa$^{-1}$ to 1 mmol m$^{-2}$ s$^{-1}$ MPa$^{-1}$ for KS.

The response of the transpiration rate to WD was analysed by calculating for each genotype the reduction ratio of the specific transpiration rate induced by WD relative to WW conditions (denoted Red$_{TrS}$). Mean Red$_{TrS}$ for 2012 and 2013 indicated that Tr$_S$ was maintained at 66% of Tr$_S$ observed under WW conditions in Grenache whereas it decreased to 53% in Syrah (Fig. 4E). This surprisingly disagreed with most studies describing Grenache as more water conservative under drought than Syrah. However, in spite of severe down-regulation of transpiration under WD in Syrah, Tr$_S$ remained quite high (e.g. 0.56 mmol m$^{-2}$ s$^{-1}$ in 2012) compared with Grenache which was less affected by WD but exhibited a limited transpiration rate (0.50 mmol m$^{-2}$ s$^{-1}$ in 2012; Fig 4B). Reduction of the transpiration rate under WD also exhibited a wide range of variation among offspring far beyond the values reported for the parents (Fig. 4E), with a significant genotypic effect (Table 1). Interestingly, the genetic variability observed for the reduction of hydraulic conductance induced by the WD (Red$_{KS}$; Fig. 4F) was of similar magnitude to Red$_{TrS}$, with also higher reduction in Syrah (lower Red$_{KS}$) than in Grenache.

### Table 1. Effects of treatment (WW, well-watered; WD, water deficit), genotype, and year on the main hydraulic traits measured in two years on the Syrah×Grenache mapping population (two parents+186 offspring) in a controlled-environment chamber

| Trait | Year | Effect of water treatment | Genetic and year effects |
|-------|------|---------------------------|--------------------------|
|       |      | WW versus WD              |                         |
|       |      | $P_s$                      |                         |
| $\Psi_M$ (MPa) | 2012 | ****                      | –0.665 ± 0.117 NS       |
|       | 2013 | ****                      | –0.658 ± 0.142 NS       |
| $\Delta \Psi$ (MPa) | 2012 | ****                      | 0.599 ± 0.117 NS        |
|       | 2013 | ****                      | 0.600 ± 0.142 NS        |
| Tr (mmol s$^{-1}$) | 2012 | ****                      | 0.11 ± 0.05             |
|       | 2013 | ****                      | 0.21 ± 0.11             |
| Tr$_S$ (mmol m$^{-2}$ s$^{-1}$) | 2012 | ****                      | 0.87 ± 0.26             |
|       | 2013 | ****                      | 0.95 ± 0.33             |
| K (mmol s$^{-1}$ MPa$^{-1}$) | 2012 | ****                      | 0.19 ± 0.11             |
|       | 2013 | ****                      | 0.37 ± 0.24             |
| KS (mmol m$^{-2}$ s$^{-1}$ MPa$^{-1}$) | 2012 | ****                      | 1.52 ± 0.58             |
|       | 2013 | ****                      | 1.66 ± 0.68             |

Means ±SD of 188 genotypic values determined on three clones of each genotype in each conditions, except WW in 2013 where two clones were measured.

Measured or derived traits were: $\Psi_M$, daytime leaf water potential; $\Delta \Psi$, difference between soil water potential and $\Psi_M$; Tr, whole-plant transpiration rate; Tr$_S$, specific transpiration rate (on a per unit leaf area basis); K, whole-plant hydraulic conductance; KS, specific hydraulic conductance; Red$_{\Psi_M}$, decrease in genotypic $\Psi_M$ from WW to WD conditions (a single value for each genotype in each year); Red$_{TrS}$ and Red$_{KS}$, the reduction ratio of Tr$_S$ and KS, respectively, induced by WD relative to WW treatment (a single value for each genotype in each year).

The significance of water treatment ($P_s$), genotype ($P_G$), and year ($P_Y$) effects is indicated as follows: *$P\leq0.05$; **$P\leq0.01$; ***$P\leq0.001$; ****$P\leq0.0001$; NS, non-significant.
mixed models and heritability

The broad-sense heritability (H²) of hydraulic traits ranged from 0 to 0.66 under the WW scenario and increased for each trait under the WD scenario up to 0.43–0.68 (Table 2). This indicated that soil drying amplified the genetic variability in hydraulic responses. For each trait, values calculated for both years were consistent. The whole-plant leaf area showed the highest H² for both years. Analysis of the whole set of data combining the two years increased H² values for all traits (Table 2), suggesting a higher power for QTL detection.

QTL analysis

QTL analysis of combined data for both years was carried out for each soil water condition. Twenty-two significant QTLs (P_G<0.05) were detected by multiyear analysis (indicated as ‘1213’ in Table 3 and Fig. 6) of all hydraulic-related traits, although with some redundancy when QTLs were detected for those traits which were derived from others by calculation (such as for ∆Ψ_M or Red_Ψ_M as compared with Ψ_M). One to four significant QTLs (P_G<0.05) were identified per trait, each accounting for 9–20% of total variance. The QTLs were located on eight chromosomes, but many co-localized on four linkage groups (LGs) (four on LG01, five on LG10, three on LG17, and four on LG18).

Four significant QTLs were detected for Ψ_M under WD on LG1 (two QTLs), LG10, and LG18, and one of them co-localized on LG18 with the QTL detected for Red_Ψ_M, both traits being indicative of (an)isohydric behaviour. One significant QTL was also detected for TrS under WD on LG17. No significant QTL was detected for the reduction of specific transpiration rate (Red_TrS) induced by the WD relative to the WW scenario.

Single-year and single-water treatment analyses revealed 32 significant QTLs (P_G<0.05). On average, 1–5 significant QTLs were detected per trait, accounting for 8–20% of the total variance. Only one significant QTL was stable at the genome level in the two years (LA, on LG18). Nevertheless, for about half of the significant QTLs detected in the multiyear analysis, a significant QTL at the same locus was detected at least in one year, combined with a putative QTL (P_df<0.05) for the other year (Table 3). These redundant QTLs between years were not repeated in Fig. 6. In only two cases, significant QTLs specifically detected in one year under one water scenario were not reported as significant or putative in the multiyear analysis (Tr WW and KS WD in 2012) (Table 3, Fig. 6).
Fig. 3. Comparison between 2012 and 2013 for mean genotypic values of hydraulics-related traits estimated under water deficit conditions for each offspring of the Syrah×Grenache mapping population. See Table 1 for abbreviations (ΨM in MPa, ∆Ψ in MPa, TrS in mmol m⁻² s⁻¹, KS in mmol m⁻² s⁻¹ MPa⁻¹, LA in m²). n=188 for each year and each trait. Pearson's determination coefficients are given, with *** indicating high statistical significance (P<10⁻⁹). Regression lines are represented in black and bisecting lines in grey.

Fig. 4. Distributions of mean genotypic values (Best Linear Unbiased Predictions; BLUPs) for hydraulic traits measured on the Syrah×Grenache mapping population. See Table 1 for abbreviations. For ΨM (A), TrS (B), and KS (C), distributions obtained under the water deficit treatment are presented for 2012 (black lines) and 2013 (grey lines) for the n=188 genotypes. For Red_ΨM (D), Red_TrS (E), and Red_KS (F), only one mean per genotype was obtained in each year, and the black line represents the distribution of the BLUP extracted from the single means of the two years of the experiment. Values for the parents are indicated with black arrows (only for 2012 in A, B, and C for the sake of clarity).
Co-localizations of QTLs and clusters of interest

The different QTL analyses resulted in identification of genomic regions of particular interest, on LG01, LG02, LG10, LG17, and LG18 (Fig 6). The multiyear QTLs detected for $\Psi_M$ tightly co-localized with QTLs for $\Delta \Psi$ (LG01, LG10, and LG18), suggesting that both traits carried similar information. This is consistent with the similar control of soil water potential which was imposed on all genotypes so that combining $\Psi_{soil}$ with $\Psi_M$ in the calculation of $\Delta \Psi$ did not introduce any substantial change in genetic architecture of these traits. A similar conclusion was obtained when analysing the difference in $\Psi_M$ between WW and WD conditions (Red_$\Psi_M$) since genetic variation was not detected under WW conditions in the multiyear analysis (Table 2). Regarding WD conditions, only one co-localization between QTLs of $\Psi_M$ and specific transpiration rate (TrS) was detected on LG10. In contrast, more co-localizations were found between QTLs of soil-to-leaf hydraulic conductance (KS) and TrS on LG01, and LG17. This could be due to the way in which KS was derived from TrS. However, some specific QTLs were also detected for KS and TrS which did not co-localize.

Finally, to examine possible genetic links between plant size and hydraulic traits, QTL detection was also performed on LA. Although this trait varied slightly among replicates with the date of measurement and between years (with larger plants in 2013 compared with 2012, Fig. 3), QTLs were detected, suggesting that genetic differences were conserved across lots of plants (blocks and years). Two QTLs were detected in the main clusters of LG17 and LG18 (Fig. 6), where they co-localized with QTLs for specific transpiration rate or specific hydraulic conductance (Fig. 6).


| Trait | Scenario | $H^2$ (2012) | $H^2$ (2013) | $H^2$ (12 + 13) |
|-------|----------|--------------|--------------|-----------------|
| Tr    | All      | -            | 0.652        | 0.717           |
|       | WD       | 0.544        | 0.6          | 0.616           |
|       | WW       | 0.476        | 0.494        | 0.519           |
| TrS   | All      | 0.677        | -            | 0.788           |
|       | WD       | 0.546        | 0.572        | 0.676           |
|       | WW       | 0.548        | 0.442        | 0.662           |
| K     | All      | -            | 0.55         | 0.618           |
|       | WD       | 0.432        | 0.549        | 0.54            |
|       | WW       | 0.459        | 0.395        | 0.4             |
| KS    | All      | 0.53         | -            | 0.725           |
|       | WD       | 0.58         | 0.534        | 0.66            |
|       | WW       | 0.41         | 0.297        | 0.465           |
| $\Psi_M$ | All | 0.486     | -            | 0.67            |
|        | WD       | 0.6          | 0.538        | 0.625           |
|        | WW       | -            | 0.232        | -               |
| $\Delta \Psi$ | All | -         | -            | 0.66            |
|        | WD       | 0.59         | 0.53         | 0.612           |
|        | WW       | -            | 0.223        | -               |
| LA    | All      | 0.672        | 0.755        | 0.823           |
| Red,$\Psi_M$ | WD - WW | -          | -            | 0.344           |
| Red,TrS | WD / WW | -          | -            | 0.292           |
| Red,KS | WD / WW | -          | -            | 0.299           |

Heritability was calculated from genotypic and residual variances obtained by fitting mixed linear models, respectively, for the WW and WD scenarios, and for the multisenario data sets (‘all’) when genotype×scenario interaction was significant.

Regarding the significant QTLs, the major effects were generally additive, originating from Shiraz ($A_S$) and/or Grenache alleles ($A_D$) and rarely associated with the dominance effect ($D$). The main QTL found for LA originated from both parents (Table 3).

### Discussion

**Phenotyping platforms facilitate the detection of genetic determinism of water relations**

This is the first study where measurements of leaf water potential have been performed together with determination of the transpiration rate on all progeny from a cross. This work showed the benefit of using a phenotyping platform. By strictly controlling the individual soil water status through the daily weighing of pots, water shortage could be imposed on woody plants with good reproducibility between genotypes and years whatever the evaporative conditions and plant development. The platform also gave access to dynamic estimates of leaf area in a non-invasive way, which was essential in the determination of specific transpiration rates.

Furthermore, accurate control of atmospheric conditions in a growth chamber made it possible to characterize water relations of all the progeny for genetic analysis. Broad-sense heritability was quite high and consistent between years for most traits. For the first time, the genetic architecture of leaf water potential was analysed in transpiring plants under WD, and 32 QTLs involved in the control of water relations were detected. This offered a unique context to dissect the genetic origin of leaf water homeostasis and to shed new light on possible mechanisms causing differences between iso- and anisohydrd behaviours.

**A genetic origin for (an)isohydrd behaviours**

Overall, the WD which was imposed in the present study on potted grapevine plantlets significantly decreased $\Psi_M$ compared with WW conditions, but with a substantial range of variability within the progeny. As a major outcome, this variability in $\Psi_M$ under WD appeared to be largely determined by the genotype. Genetic variability in $\Psi_M$ under WW conditions was not detected. Therefore, genetic variability in $\Psi_M$ under WD was indicative of the differences between more isohydrd (high $\Psi_M$ under WD) and more anisohydrd (low $\Psi_M$ under WD) behaviours. The wide range of genetic variation observed in the progeny compared with the parents suggested that several heterozygous QTLs were borne by the parental lines and segregated within the progeny. These conditions were favourable for the detection of QTLs governing the complex genetic determinism of the studied traits.

It should be noted that $\Psi_M$ primarily depends on both soil WD and evaporative conditions, which were chosen as moderate in this study. The WD treatment corresponded to a moderate decrease in $\Psi_{swi} (-0.15\text{MPa for all the progeny})$ compared with non-irrigated field conditions where pre-dawn leaf water potential (a proxy for $\Psi_{swi}$) frequently decreases down to $-0.6\text{MPa}$ (Prieto et al., 2010). Evaporative conditions in the controlled environment chamber were also chosen as intermediate by setting the VPD close to 2 kPa which has been shown to trigger intermediate stomatal responses in several species (Sinclair et al., 2008; Gholipoor et al., 2013; Ocheltree et al., 2013), whereas a higher VPD induces severe stomatal closure (Monteith, 1995). In the experiments carried out here, the VPD was high enough to induce a genetically variable reduction in transpiration rate (Fig. 2, Table 1) and thus, possibly, in $\Psi_M$, but not too excessive to avoid severe limitations in the transpiration rate, which could have hindered the detection of genetic variation.

Genetic control of $\Psi_M$ under stabilized evaporative and soil WD conditions was confirmed by the detection of several underlying QTLs on the consensus map. Four QTLs for $\Psi_M$ under WD were detected in the multiyear analysis, each one explaining at least 11% of total variability. QTLs detected for $\Psi_M$ under WD were most often highly significant in one year and putative in the other year. Difference between parental cultivars was modest and was dramatically transgressed within the progeny. This result is consistent with previous studies (Prieto et al., 2010; Pou et al., 2012), showing that the (an)isohydrd reputations of Grenache and Syrah can be considered as overstated as compared with other cultivars. This also indicates possible genetic gains as regards leaf water homeostasis under drought.
Table 3. Significant quantitative trait loci (QTLs) detected on the consensus map of the Syrah×Grenache mapping population for the hydraulics-related traits (abbreviations as in Table 1) and leaf area (LA)

Results of multiyear (‘12 + 13’) and single-year (‘12’ and ‘13’) analyses are presented for well-watered (WW) and water deficit (WD) scenarios, and for the multisenario data sets (‘all’)
Fig. 6. Localization of the most significant quantitative trait loci (QTLs) involved in the genetic determinism of the hydraulic-related traits in a Syrah×Grenache mapping population. Central marks on the vertical bars indicate the position L on each linkage group (LG01–LG18) of the consensus map where the maximum LOD score was observed (bold mark for multiyear QTLs and thin mark for 1-year QTLs). Vertical bars are for the LOD–1 confidence intervals around L. QTL names indicate the trait and the conditions (water treatment and years) retained for QTL analysis. QTLs were detected for: $\Psi_M$, daytime leaf water potential; Tr and TrS whole-plant and specific transpiration rates, respectively; K and KS, whole-plant and specific hydraulic conductances, respectively; LA, whole-plant leaf area. QTLs represented here are mainly those detected on the multiyear data set (indicated as '1213' in the QTL name). When a QTL was also detected in one specific year as highly significant, the corresponding year is underlined in the QTL name. Highly significant QTLs detected in 1-year but not in multiyear analysis are also represented and identified with the corresponding year '12' or '13' in the QTL name. The middle part of the QTL name indicates either ‘WD’, ‘WW’, or ‘all’ when a QTL was detected respectively using water deficit, well-watered, or all water treatment data sets. (This figure is available in colour at JXB online.)
The transpiration rate was reduced by about a half by the mild WD conditions applied in the present study. Given that measurements were carried out in a strictly controlled environment, any change in transpiration rate could be ascribed to a change in stomatal conductance provided that the transpiration rate was expressed on a leaf area basis (i.e. by calculating the specific rate TrS). However, it should be noted that stomatal conductance may not be the only factor influencing transpiration and possibly involved in the transpiration response to WD. First, cuticular conductance should be considered. However, it was unlikely that the cuticle changed during the 7 days of soil drying preceding measurements. Similarly, boundary layer conductance in the controlled-environment chamber was mostly conserved between WW and WD conditions. In spite of strict control of atmospheric conditions in the chamber, TrS was slightly but significantly higher in 2013 compared with 2012 (regardless of water regime). This could be explained by the lower plant density in the growth chamber that was imposed in 2013 to facilitate leaf sampling. The reduction of plant density by about one-third in 2013 may have slightly increased the boundary layer conductance and probably resulted in more leaves exposed to direct light (which were selected for \( \Psi_M \) measurements in all cases). However, evaporative conditions were similar for WW and WD plants within each year. Eventually, within each year, only variations in leaf temperature induced by the reduction in transpiration rate under WD may have slightly distorted the relationship between specific transpiration rate and stomatal conductance. Stomatal closure in response to soil WD was therefore the primary cause of reduction in TrS induced by WD. A wide range of variation was found in the progeny, with slight reduction in transpiration rate for some genotypes (Red\(_{\text{TrS}}\)=0.78) down to stronger reduction for others (Red\(_{\text{TrS}}\)=0.42). A similar range of genetic variation in transpiration rate under WD was observed in scions grafted on a rootstock progeny (Marguerit et al., 2012).

Variation in drought-induced stomatal closure, as approximated here by the reduction in transpiration rate, is thought to be at the origin of the contrasted behaviours between iso- and anisohydric plants (Buckley, 2005). This proposal, which has never been examined on a wide genetic scale, is challenged in this study by the absence of tight correlation under WD between \( \Psi_M \) (or Red\(_{\Psi_M}\)) and specific transpiration rate (or Red\(_{\text{TrS}}\)) within the progeny (Fig. 5). Even more puzzling, the corresponding correlations, although loose, were positive. This was not expected if a strong reduction in TrS (low Red\(_{\text{TrS}}\) and low TrS) associated with stomatal closure were to play a role in the maintenance of high \( \Psi_M \).

In other words, the way in which \( \Psi_M \) was decreased more or less severely by soil drying cannot be considered as simply determined by the range of variation in transpiration rate that was observed within the progeny. This is corroborated by the present results on QTL detection. Only one co-localization was found between a QTL detected for \( \Psi_M \) and specific transpiration rate (LG10), whereas other QTLs were specifically detected for \( \Psi_M \) on LG01 and LG18. This suggests, again, that the genetic controls of \( \Psi_M \) and stomatal regulation only partly share common determinisms and may also rely on independent mechanisms. It should be noted that heritability for reduction in transpiration rates (Red\(_{\text{TrS}}\)) was quite low in this study. Red\(_{\text{TrS}}\) was calculated as one single value for each genotype in each year and could not be determined with replications on individual plants which were exclusively characterized under either WW or WD treatment. This could explain why no QTL was detected for this trait. QTLs detected for specific transpiration were not systematically significant over the two years, as was the case in similar studies (Marguerit et al., 2012). The most likely explanation is the high number of QTLs which are expected to control the transpiration rate in agreement with the large amount of genes identified as controlling stomatal differentiation (Barton, 2007) and functioning (Ward et al., 2009). In such conditions, individual QTLs have weak effects and their detection becomes tricky.

Besides the role of stomata, it makes sense to question the contribution of hydraulic conductance in the control of leaf water homeostasis (Cochard et al., 2002) since hydraulic conductance (from soil to leaves as considered in this study) determines the value of \( \Psi_M \) for a given value of transpiration rate and also because changes in hydraulic conductance may influence the stomatal control of transpiration (Pantin et al., 2013). Within the Syrah×Grenache progeny, under WD, specific hydraulic conductance showed a high heritability and many QTLs were detected. Interestingly, although the calculation of KS was based on \( \Psi_M \) and therefore partly shared the same source of variation, only one QTL for KS co-localized with one of the QTLs detected for \( \Psi_M \) under WD (LG18). This suggests that, as for transpiration rate, genetic variability in hydraulic conductance may participate in the control of (an)isohydric behaviours but cannot fully account for them.

Over the whole grapevine progeny, WD reduced soil-to-leaf hydraulic conductance by about a half, but with contrasted responses within the progeny. This raises questions on the physiological origin of this genetic variability. Measurements in field conditions have already revealed rapid and severe impacts of soil drying on soil-to-leaf hydraulic conductance in grapevine cultivars, specifically in the daytime (Zufferey et al., 2011). In the conditions used here, soil drying developed in ~1 week before measurements so that plant architecture (vessel diameter or leaf venation) was not greatly modified compared with WW plants during this short, terminal period of differential treatment. Moreover, WD treatment caused only a moderate decrease in water potential which was unlikely to have induced substantial cavitation in the xylem pathway. Therefore, the observed reduction in specific hydraulic conductance more probably resulted from rapid changes in water transport capacity such as those already revealed in grapevine with the modification of aquaporin expression in roots (Vandeleur et al., 2009) or leaves (Pou et al., 2013).
The soil-to-root pathway which represents a major resistance to water flow (Tramontini et al., 2013, in grapevine) is very sensitive to changes in soil water content (van Genuchten et al., 1980), and could have also contributed to the reduction in hydraulic conductance and to genetic variation within the progeny in the present study. However, the same rootstock (110R) was used for all offspring. Genetic differences originating in leaves rather than roots are more consistent with a variation in scions as involved in this study and with current knowledge on the effects of drought-induced signals such as abscisic acid on aquaporin activity in the sheaths of leaf veins (Shatil-Cohen et al., 2011). Specifically, changes in some aquaporin activity in leaves induced by water stress have been correlated with changes in leaf hydraulic conductance (Pou et al., 2013). However, it should also be envisaged that genetic variation of the scion was possibly accompanied by differences in rootstock development and root water transport activity, which merits further studies.

**Conclusions**

The genetic analysis of water relations in the Syrah×Grenache progeny revealed that reduction in the transpiration rate induced by soil drying was not the only factor to be considered for the maintenance of leaf water potential in the daytime. Determinism of (an)isohydry should be sought in deviations from the balance between water supply capacity (hydraulic conductance) and stomatal control of transpiration. From a physiological point of view, this suggestion is consistent with the proposal that diversity in (an)isohydric behaviours originates in mechanisms uncoupling stomata from direct hydraulic control (Tardieu and Simonneau, 1998). From a genetic point of view, variability in (an)isohydric behaviour most probably rests on QTLs detected for the specific transpiration rate but not for specific hydraulic conductance (such as on LG10) and vice versa (LG13 and LG18). The present study also revealed some genetic determinism of \( \Psi_M \) variation under WD that appeared to be independent on both specific transpiration rate and specific hydraulic conductance (LG01). Recombining or selecting allelic variations in these QTLs appears to be independent on both specific transpiration rate and specific hydraulic conductance (LG01). Recombining or selecting allelic variations in these QTLs appears to be a promising way to build cultivars with varying (an)isohydric behaviours.

**Supplementary data**

Supplementary data are available at JXB online.  
**Figure S1.** Mean shoot growth response of the main axis of cv. Grenache plants to soil water content.  
**Figure S2.** Comparison between measured and predicted plant biomass and leaf area.  
**Figure S3.** Relationship between soil water content (SWC) and soil water potential (\( \Psi_{soil} \)).  
**Figure S4.** Evolution of hourly transpiration rate during a 6h period after switching on the lights in a controlled-environment chamber and relationship between the transpiration rate calculated over the whole 6h light period and the 2h period when leaf water potential was determined.

**Table S1.** Mixed models selected for the extraction of BLUPs of genetic values.  
**Table S2.** Putative quantitative trait loci (QTLs) (\( F_{\text{chr}} < 0.05 \)) detected on the consensus map of the Syrah×Grenache progeny for the hydraulics-related traits and leaf area measured in the Phenotyping Platform PhenoArch.

**Acknowledgements**

Claude Morel, Suppawadee Sittichai, Victorien Taudou, Cédric Ouvray, and Mathieu Moreno took an active part in measurements of the water relations and Antonin Grau in platform management. Denis Vile provided useful help in the estimation of individual leaf areas. This work was supported by the French program ANR-09-GENM-024-002. AC-L received a PhD grant from the French Government.

**References**

Abiola O, Angel JM, Avner P, et al. 2003. The nature and identification of quantitative trait loci: a community’s view. Nature Reviews Genetics 4, 911–916.

Adam-Blondon AF, Roux C, Claux D, Butterlin G, Merdinoglu D, This P. 2004. Mapping 245 SSR markers on the Vitis vinifera genome: a tool for grape genetics. Theoretical and Applied Genetics 106, 1017–1027.

Ambrose AR, Sillett SC, Dawson E. 2009. Effects of tree height on branch hydraulic conductance, leaf structure and gas exchange in California redwoods. Plant, Cell and Environment 32, 743–757.

Barton MK. 2007. Making holes in leaves: promoting cell state transitions in stomatal development. The Plant Cell 19, 1140–1143.

Borel C, Simonneau T, This D, Tardieu F. 1997. Controls of stomatal conductance and ABA concentration in the xylem sap of barley lines of contrasting genetic origins. Australian Journal of Plant Physiology 24, 607–615.

Buckley TN. 2005. The control of stomata by water balance. New Phytologist 168, 275–292.

Chaves M, Zarrour O, Francisco R, Costa JM, Santos T, Regalado AP, Rodrigues ML, Lopes CM. 2010. Grapevine under deficit irrigation: hints from physiological and molecular data. Annals of Botany 105, 661–676.

Churchill GA, Doerge RW. 1994. Empirical threshold values for quantitative trait mapping. Genetics 138, 963–971.

Cochard H, Coll L, Roux XL, Ameglio T. 2002. Unravelling the effects of plant hydraulic on stomatal closure during water stress in walnut. Plant Physiology 128, 282–290.

Franks PJ, Drake PL, Froend R. 2007. Anisohydric but isohydrodynamic: seasonally constant plant water potential gradient explained by a stomatal control mechanism incorporating variable plant hydraulic conductance. Plant, Cell and Environment 30, 19–30.

Ghollipoor M, Choudhary S, Sinclair TR, Messina CD, Cooper M. 2013. Transpiration response of maize hybrids to atmospheric vapour pressure deficit. Journal of Agronomy and Crop Science 199, 155–160.

Huang YF, Doligez A, Fournier-Level A, et al. 2012. Dissecting genetic architecture of grape proanthocyanidin composition through quantitative trait locus mapping. BMC Plant Biology 12, 1–19.

Jansen RC. 1993. Interval mapping of multiple quantitative trait loci. Genetics 135, 205–211.

Jansen RC, Stam P. 1994. High resolution of quantitative traits into trait locus mapping. Genetics 139, 153–160.

Margueret E, Brendel O, Lebon E, Van Leeuwen C, Ollat N. 2012. Rootstock control of scion transpiration and its acclimation to water deficit are controlled by different genes. New Phytologist 194, 416–429.

McDowell N, Pockman WT, Allen CD, et al. 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytologist 178, 719–739.

Monteith JL. 1995. A reinterpritation of stomatal responses to humidity. Plant, Cell and Environment 18, 357–364.
Ocheltree TW, Nippert JB, Prasad PV. 2013. Stomatal responses to changes in vapor pressure deficit reflect tissue-specific differences in hydraulic conductance. *Plant, Cell and Environment* **37**, 132–139.

Pantin F, Monnet F, Jannaud D, Costa JM, Renaud J, Muller B, Simonneau T, Genty B. 2013. The dual effect of abscisic acid on stomata. *New Phytologist* **197**, 65–72.

Pou A, Medrano H, Tomas M, Martorell S, Ribas-Carbo M, Flexas J. 2012. Anisohydric behaviour in grapevines results in better performance under moderate water stress and recovery than isohydric behaviour. *Plant and Soil* **359**, 335–349.

Pou A, Medrano H, Flexas J, Tyerman SD. 2013. A putative role for TIP and PIP aquaporins in dynamics of leaf hydraulic and stomatal conductances in grapevine under water stress and re-watering. *Plant, Cell and Environment* **36**, 828–843.

Prieto J, Lebon E, Ojeda H. 2010. Stomatal behavior of different grapevine cvs in response to soil water status and air water vapor pressure deficit. *Journal International des Sciences de la Vigne et du Vin* **44**, 9–20.

R Development Core Team. 2012. *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.

Royston P. 1995. Remark AS R94: a remark on Algorithm AS 181: the W test for normality. *Applied Statistics* **44**, 547–551.

Shatil-Cohen A, Attia Z, Moshelion M. 2011. Bundle-sheath cell regulation of xylem-mesophyll water transport via aquaporins under drought stress: a target of xylem-borne ABA. *The Plant Journal* **67**, 72–80.

Sinclair TR, Zwieniecki MA, Holbrook NM. 2008. Low leaf hydraulic conductance associated with drought tolerance in soybean. *Physiologia Plantarum* **132**, 446–451.

Soar CJ, Speirs J, Maffei SM, Penrose AB, McCarthy MG, Loveys BR. 2006. Grape vine varieties Shiraz and Grenache differ in their stomatal response to VPD; apparent links with ABA physiology and gene expression in leaf tissue. *Australian Journal of Grape and Wine Research* **12**, 2–12.

Tardieu F, Davies WJ. 1992. Stomatal response to ABA is a function of current plant water status. *Plant Physiology* **98**, 540–545.

Tardieu F, Lafarge T, Simonneau T. 1996. Stomatal control by fed or endogenous xylem ABA in sunflower: interpretation of observed correlations between leaf water potential and stomatal conductance. *Plant, Cell and Environment* **19**, 75–84.

Tardieu F, Simonneau T. 1998. Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydic and anisohydric behaviours. *Journal of Experimental Botany* **49**, 419–432.

Tramontini S, van Leeuwen C, Domec J-C, Destrac-Irvine A, Bastau C, Vitali M, Mosbach-Schulz O, Livosolo C. 2013. Impact of soil texture and water availability on the hydraulic control of plant and grape-berry development. *Plant and Soil* **368**, 215–230.

Tsuda M, Tyree MT. 2000. Plant hydraulic conductance measured by the high pressure flow meter in crop plants. *Journal of Experimental Botany* **51**, 823–828.

Vandeleur R, Mayo G, Shelden M, Gillham M, Kaiser B, Tyerman S. 2009. The role of plasma membrane intrinsic protein aquaporins in water transport through roots: diurnal and drought stress responses reveal different strategies between isohydic and anisohydric cultivars of grapevine. *Plant Physiology* **149**, 445–460.

Van Gennuchten MT. 1980. A closed-form equation for predicting the hydraulic conductivity of unsaturated soils. *Soil Science Society of America Journal* **44**, 882–898.

Van Ooijen JW, Maliepaard C. 1996. *MapQTL™ version 4.0: software for the calculation of QTL positions on genetic maps*. Wageningen: CPRO-DLO.

Ward JM, Fraser P, Schroeder JI. 2009. Plant ion channels: gene families, physiology, and functional genomics analyses. *Annual Review of Physiology* **71**, 59–82.

Zufferey Y, Cochard H, Ameglio T, Spring JL, Viret O. 2011. Diurnal cycles of embolism formation and repair in petioles of grapevine (*Vitis vinifera cv. Chasselas*). *Journal of Experimental Botany* **62**, 3885–3894.