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Factors influencing stomatal conductance in response to water availability in grapevine: a meta-analysis

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The main factors regulating grapevine response to decreasing water availability were assessed under statistical support using published data related to leaf water relations in an extensive range of scion and rootstock genotypes.

Matching Ψ_{leaf} and g_s data were collected from peer-reviewed literature with associated information. The resulting database contained 718 data points from 26 different *Vitis vinifera* varieties investigated as scions, 15 non-*V.vinifera* rootstock genotypes and 11 own-rooted *V. vinifera* varieties. Linearized data were analyzed using the univariate general linear model (GLM) with factorial design including biological (scion and rootstock genotypes), methodological and environmental (soil) fixed factors.

The first GLM performed on the whole database explained 82.4% of the variability in data distribution having the rootstock genotype the greatest contribution to variability (19.1%) followed by the scion genotype (16.2%). A classification of scions and rootstocks according to their mean predicted g_s in response to moderate water stress was generated. This model also revealed that g_s data obtained using a porometer were in average 2.1 times higher than using an infra-red gas analyser. The effect of soil water-holding properties was evaluated in a second analysis on a restricted database and showed a scion-dependant effect, which was dominant over rootstock effect, in predicting g_s values.

Overall the results suggest that a continuum exists in the range of stomatal sensitivities to water stress in *V. vinifera*, rather than an isohydric- anisohydric dichotomy, that is further enriched by the diversity of scion-rootstock combinations and their interaction with different soils.

Key words: scion, rootstock, isohydric, anisohydric, *Vitis*, water potential

Abbreviations

ABA, absisic acid; $A_{N,}$, net assimilation of CO_2; $g_{s,}$, stomatal conductance; IRGA, infra-red gas analyser; PRD, partial root drying; WUE, water use efficiency; Ψ_{leaf}, leaf water potential; Ψ_{stem}, stem water potential.

Introduction

Further decrease in water availability is predicted in some important viticulture areas such as the Mediterranean basin as a consequence of increased atmospheric green gas emissions and associated rise in surface temperatures (IPCC 2014). These climatic changes would lead to increased atmospheric water demand and consequently to an increased rate of crop evapotranspiration and soil water depletion. Excessive water limitation impairs plant growth and consequently the amount of exposed leaf area, compromising the maturation of fruit and on the long term the carbon balance of the crop and its lifespan. These represent a major threat to the wine industry sector for which major agronomical and enological adaptations will be required to sustain its activity. Although irrigation could be considered as a solving option, it is not a sustainable one in many viticulture settings.

Efforts have been made to better understand the mechanisms of water use efficiency (WUE) in grapevine (Flexas et al. 2016). Adapting viticulture practices based on the physiological mechanisms regulating grapevine's water consumption could offer a biological alternative to irrigation. The intrinsic WUE (WUE_i) represents the amount of carbon assimilated under the form of CO_2 (A_N) in exchange of water molecules and mainly depends on stomatal conductance (g_s). WUE_i (A_N/g_s) is higher at low g_s and follows an inverse non-linear relationship but was shown to vary greatly among cultivars (Tomás et al. 2014). This suggests that grapevine genotype with a propensity for reduced stomatal conductance could perform better in low water availability conditions (Ferrandino and Lovisolo 2014).

Indeed the *Vitis vinifera* species possess this singularity to show different strategic behaviours in response to drought among its varieties (Schultz 2003). This has made *V. vinifera* a model-plant to study the physiologic mechanisms of drought resistance, which are described as mechanisms of stress "tolerance" or "avoidance". Some cultivars are described as drought avoidant or "near-isohydric" because they close stomata early in response to decreased soil water content in order to prevent water loss and maintain their organs' water potential (Ψ) stable, similar to those of well-watered plants (Schultz 2003). Other cultivars described as drought tolerant or "near-anisohydric" show higher tolerance to drops in plant

organ Ψ, closing stomata at levels well under those of well-watered plants. We have recently shown that aquaporins confer higher level of anisohydry by controlling leaf hydraulic capacitance (Vitali et al. 2016), and aquaporin expression is known to be under genetic control in different grapevine cultivars. Although iso/anisohydric control of water stress has been reproduced in a number of studies for given cultivars (e.g. Grenache and Syrah as near-iso- and near-anisohydric varieties, respectively (Gerzon et al. 2015, Schultz 2003, Soar et al. 2006b, Tramontini et al. 2013b) it has been difficult to classify grapevine cultivars as strictly near-iso- or near-anisohydric (Chaves et al. 2010). This suggests that other factors influence the response of grapevine cultivar to water stress.

Water uptake is influenced by root architecture (Ollat et al. 2015). Grapevine root growth plasticity in response to soil moisture determines when and where roots capture water and nutrients (Comas et al. 2010). In addition, the root system impacts on grapevine evapotranspiration rate through both chemical and hydraulic root-to-shoot signals, therefore acting distally on the guard cells that constitute the stomata (Zhang et al. 2016). Importantly, today's grapevines are for the most grafted onto rootstocks of American genotypes resistant to Phylloxera. These different rootstock genotypes have been shown to differentially impact on scion's performance in terms of water consumption (Marguerit et al. 2012, Ollat et al. 2015, Berdeja et al. 2015). For instance, varying absisic acid (ABA) concentrations, a hormone produced by drying root cells (chemical signal), were found in the xylem sap of the same scion grafted over different rootstocks and was associated to stomatal conductance (Soar et al. 2006a). In addition, embolisms that contribute to reduce root hydraulic conductance occur in a wide range of proportions (5-75%) across the different rootstock genotypes (Lovisolo et al. 2008b). Embolisms are thought to act as hydraulic signals inducing the closure of stomata in order to limit water use by the plant and protect against the propagation of low xylem tension to the stem. While soil is drying out, soil water potential drops, which is the reason for the plant water potential to fall in order to maintain water uptake. Thus embolism is the most severe form of hydraulic signal along the hydraulic continuum. Susceptibility to embolism in rootstocks was shown to be associated to root system architecture and aquaporin

contribution to water flow during water stress (Alsina et al. 2011, Gambetta et al. 2012, Lovisolo et al. 2008b). Thus root hydraulic conductivity and the property to induce vigour in scion vary among rootstocks of different origin i.e. crossings of *V. rupestris*, a xerophilic species, and crossings of *V. riparia*, a mesophilic species. Taken together these observations suggest that the rootstock genotype can modulate the intrinsic response of scion to water stress through both ABA and hydraulic signalling. This hypothesis is supported by recent findings where drought-resistant (140Ru, *V. berlandieri x V. rupestris*) and drought-sensitive rootstocks (SO4, *V. berlandieri x V. riparia*) can respectively shift the threshold of stomatal closure towards lower (140Ru) or upper (SO4) Ψ_{leaf} in either a near-iso- (*V. vinifera* cv. Grenache) or near-anisohydric cultivars (*V. vinifera* cv. Syrah) in a water-stress situation (Tramontini et al. 2013b).

To further complicate the picture, soil texture is apparently able to influence the mechanisms regulating the response of grapevine to drought, as water-retaining soil (clay-rich) was shown to decrease stomatal aperture (Tramontini et al. 2013a) as well as the extent of embolism formation and to increase ABA concentrations in leaves of both near-iso- and near-anisohydric cultivars (Tramontini et al. 2014). The soil water potential depends on the soil properties according to water retention soil dynamics. While for the same water content, the water potential is higher for sandy soils than for clay soils, the water holding capacity is lower and thus sand is drying out faster than clay (Draye et al. 2010). Hence a better matching of grapevine scion and rootstock varieties to vineyard sites based on these parameters represents a biological alternative for adaptation to such changing environments.

However the concomitant influence of soil, rootstock and scion on the overall grapevine tolerance to drought is rather difficult to predict and, as recently addressed by Lovisolo and colleagues (Lovisolo et al. 2016), likely triggers complex and interacting mechanisms involving both hormonal and hydraulic signals. In order to gather the current information and reveal the main factors regulating grapevine response to decreasing water availability, we reviewed and questioned published data related to leaf water relations in an extensive range of scion and rootstock genotypes under statistical support. We hypothesized that the genotype background of rootstocks can influence the stomatal sensitivity of the scion by

modifying the relationship between leaf water potential and stomatal conductance. We also expected the soil water-holding properties to interact with this relationship.

Materials and methods

Physiological parameters used for the meta-analysis

Leaf water potential (Ψ_{leaf}): Ψ in leaves results from both pressure potential and osmotic potential. Ψ_{leaf} is measured using a leaf pressure chamber and is considered to be an integrative indicator of plant water status: the climatic demand in water, plant water availability, stomatal conductance as well as whole system hydraulic conductivity are taken into consideration using this measurement (Saurin et al. 2014). Only data associated with manifest water stress in grapevine were used ($\Psi_{leaf} < -0.5$ MPa), according to Lovisolo et al. (2010) and Chaves et al. (2010). The authors consider predawn Ψ_{leaf} more of an agronomic tool to monitor plant water status associated to Ψ_{soil} than a parameter for assessing physiological response to drought and therefore was not collected. Ψ_{stem} data were collected in a separate database and will be treated in an ulterior manuscript.

Stomatal conductance (g_s): stomatal aperture is regulated by light, vapour pressure deficit (VPD) (Perrone et al. 2012) as well as water stress through both hydraulic (Chitarra et al. 2014, Pou et al. 2013) and chemical signals ABA (Lovisolo et al. 2002) and pH (Li et al. 2011, Rodrigues et al. 2008). Under mild to moderate water stress, g_s reduction is the earliest response (Chaves et al. 2003, Medrano et al. 2002) and has been identified in grapevine as a suitable parameter to detect the degree of water stress (Cifre et al. 2005, Medrano et al. 2002). Stomatal conductance is measured by evaluating either the water vapour diffusion from the leaf to a humidity sensor using a porometer (Pearcy et al. 1989) or both water and CO_2 diffusion from the leaf according to their infrared absorption wavelength using an infrared gas analyser (IRGA).

Literature review and selection criteria

Data were collected from articles published in peer-reviewed scientific journals, conference proceedings or PhD theses. The search was performed either by keywords- (one or several of the following: grapevine, *Vitis vinifera*, rootstock, water stress, leaf water potential, stomatal conductance), author- or cross-reference-based search on various databases (Google scholar, Science Direct, Scopus, Pubmed). Over 300 publications were reviewed and the selection was based on the following criteria: (1) the study of *Vitis vinifera* scion(s) own-rooted or grafted on rootstocks having either *V. riparia* or *V. rupestris* as a parent genotype. For comparison purposes, studies in which measurements were performed directly on rootstock genotypes were also collected and; (2) the presence of both Ψ_{leaf} and g_s values matched in time (+/- < 2 h delay between parameter measurement), either under numerical or graphed values obtained from measurements performed seasonally or diurnally.

Data were collected from studies taking place either in a field or a greenhouse experimental setting. Grapevines that were grown in pots whether in the field or in the greenhouse were assigned as "potted" grapevines considering the effect of root growth restriction. Data from vines that were irrigated using a partial root drying (PRD) system or vines that were mutilated (e.g. root excision) or submitted to external physical modification (e.g. plastic cover, shading nets, pressure collar, etc.) were rejected.

Ψ_{leaf} and g_s values retrieval from publications

For data under graphical representation, ImageJ Software (Rasband) was used to retrieve values. Prior to measurements, the scale was set according to each parameter axis range and the XY coordinates (for means) were exported to spreadsheet software. When parameters were plotted as single data per grapevine instead of means, values were averaged as following: briefly, data were ranked according to Ψ_{leaf}, with their corresponding g_s data, and grouped with respect to specified *n* assuming that grapevines with similar Ψ_{leaf} were assessed at the same time point. For g_s values expressed in cm s^{-1} (i.e. cm^3 cm^{-2} s^{-1}) referring to water vapour gradients across the leaf-air barrier, a conversion to molar units was applied according to Equation 1 from (Pearcy et al. 1989):

$$g_{mol\ m\text{-}2\ s\text{-}1} = g_{cm\ s\text{-}1}\ 0.446 \left(\frac{273}{T+273}\right)\left(\frac{P}{101.3}\right) \tag{1}$$

where T is the temperature in °C, and P is the atmospheric pressure in kPa. Data from three

references needed conversion to molar units for which reported leaf temperatures and normal atmospheric

pressure were used (Frieman 1982, Kliewer 1983, Loveys 1984, Table S1, see supporting material). The

number of data retrieved per variety depended mainly on the minimum number of data furnished in the

publication. However when numerous data were available, the selection was based on the optimum

number of data required to best cover the variation in Ψ and g_s.

Supplemental information collected in the database

The following information were associated to the data in the database, when available: n of vines

studied, n of leaves per vine used for measurements, grapevine age, use of irrigation, training system, time

course of measurements (diurnal or seasonal), soil description, use of potted versus field-grown vines, and

method for measuring g_s. The soil descriptions were further evaluated following the advices from a soil

scientist in order to determine their water-retaining or -draining properties, which were tightly related to

the clay content (Table S2). Indeed it is clear that water potential in soil influences stomatal conductance,

rather than the soil type itself. A well-watered sand can lead to high stomatal conductance, while a dry

loamy soil can also lead to an early onset of stomatal closure. In this sense the soil type refers to its water-

holding capacity and related water retention curves.

Statistical analysis

Data were analysed using the univariate general linear model with factorial design on the IBM®

SPSS® Statistic Software Version 21. The g_s data was used as dependent variable. The statistical model

considered Ψ_{leaf} as covariate and biological (scion and rootstock genotypes), methodological (pot or field

study, diurnal or seasonal monitoring of physiological parameters; and method for g_s assessment) and

environmental (soil) effects as fixed factors. Assumptions were: (1) linearity of the transformed data; (2)

normality and independence of the transformed data residuals; (3) equality of error variances. Type III sum of squares hypothesis-testing method was used to account for unbalanced design. The normality of residuals was assessed using Q-Q plot analysis and both Shapiro-Wilk and Kolmogorov-Smirnov. In order to meet the assumptions, lognormal transformation of g_s was first tested but did not report positive effect. The BOX COX transformation using the power of 0.4 (g_s^0.4) according to the SPSS procedure was finally successful in allowing transformed variables to meet assumptions. In addition, values outside the 95% of normal distribution were considered outliers ($n = 17$) and were removed from the dataset.

The statistical model that was used in a first analysis considering the entire database is described in Equation 2 as following:

$$g_s\text{^}0.4 = \text{Intercept} + B_{\text{Scion genotype}} + B_{\text{Rootstock genotype}} + B_{\text{Study setting (Pot or field)}} +$$

$$B_{\text{gs assessment method (IRGA or porometer)}} + B_{\text{Time frame (diurnal or seasonal)}} + B_{\text{Scion genotype*Rootstock}} \quad (2)$$

$$\text{genotype} + B_{\Psi\text{leaf}}*\Psi_{\text{leaf}}\,(MPa) + B_{\text{Scion genotype}}*\Psi_{\text{leaf}}\,(MPa) + B_{\text{Rootstock genotype}}*\Psi_{\text{leaf}}$$

$$(MPa) + B_{\text{Scion genotype*Rootstock genotype}}*\Psi_{\text{leaf}}\,(MPa)$$

where B is the specific coefficient of each fixed factor or interaction, and Ψ_{leaf} (MPa) is the value of Ψ_{leaf} in MPa (covariate) to be multiplied by its own coefficient or the coefficient of the factor or interaction having a significant interaction with Ψ_{leaf}.

The effect of soil water holding properties on stomatal sensitivity was assessed in a second analysis restricted to 3 scions (3 levels: "Cabernet sauvignon", "Syrah" and "Tempranillo") for which all-3 soil types (3 levels: "Draining", "Intermediate" and "Retaining") were available in the database. Rootstocks were grouped under their genotype families (3 levels: "*V. riparia*", "*V. rupestris*" or "*V. Vinifera*") to increase the power of the analysis and assess the effect of rootstock genotype background as performed by

(Lovisolo et al. 2008b). The same methodological factors as in the first analysis were also taken into account. Thus the second statistical model is described in Equation 3 and included:

$$g_s{}^{\wedge}0.4 = \text{Intercept} + B_{\text{Scion genotype}} + B_{\text{Rootstock genotype family}} + B_{\text{Study setting (Pot or field)}} +$$

$$B_{\text{gs assessment method (IRGA or porometer)}} + B_{\text{Time frame (diurnal or seasonal)}} + B_{\text{Soil type}} + \qquad (3)$$

$$B_{\Psi\,\text{leaf}} * \Psi_{\text{leaf}}\ (\text{MPa}) + B_{\text{Soil type * Scion genotype}} + B_{\text{Soil type * Rootstock genotype family}}$$

The data were weighted according to the *n* number of vines used for the measurements of g_s and Ψ. If *n* was different between parameters, the smaller one was used. Pairwise comparisons of estimate means were performed using the Bonferroni's confidence interval adjustment for multiple comparisons. Predicted values of g_s were used to generate graphs using the same software.

Results

Data description

  The database used for analysis contained 718 data points obtained from 40 references published between 1982 and 2014 (Table S1). Twenty-six (26) different *V. vinifera* varieties were represented in addition to 2 rootstocks investigated as scions (110 Richter (*V. rupestris x V. berlandieri*); 101-14 Mgt (*V. riparia x V. rupestris*)). The number of data retrieved per variety per publication varied between 2-108 averaging 11.3. Syrah was the most represented scion (195 data, from 10 publications) followed by Tempranillo (89 data, from 6 publications), Cabernet Sauvignon (55 data, from 7 publications) and Grenache (52 data, from 5 publications). As for rootstocks of non-*V.vinifera* genotypes, a total of 15 were represented in the database while 11 own-rooted *V. vinifera* varieties were included. The most represented in terms of number of data was 110R (163 data, from 10 publications), followed by 140Ru (88 data, from 4 publications), 1103P (87 data, from 7 publications), and Teleki 5C (60 data, from 4 publications).

Modelling of the Ψ_{leaf}/g_s database: effect of biological and methodological factors in predicting g_s

  Values of g_s were linearized using the 0.4 power of g_s (Fig. 1A, B) and submitted to the general linear model. The normality of residual values (differences between observed and estimated values of g_s) resulting from the model is depicted using the normal Q-Q plot (Fig. 1C). Overall, the model explained 82.4% of the variation in data distribution ($r^2 = 0.824$) with all factors explaining a significant proportion of the variability expressed using the partial η^2 (Table 1), with the rootstock genotype having the greatest contribution (19.1%, $P < 0.0001$) followed by the scion genotype (16.2%, $P < 0.0001$). A factor having a significant effect indicates that it modifies the position of the Ψ_{leaf}-g_s curve along the vertical axis i.e. the value of g_s for this factor (e.g. scion) changes significantly according to its levels (e.g. Syrah, Grenache, etc.) for a given Ψ_{leaf}. The significant interaction between Ψ_{leaf} and scion genotype implies that the slope of the Ψ_{leaf}-g_s curve varies according to the different scions as shown in Fig. 1D, depicting the values of g_s

predicted by the model. Similarly a significant interaction between rootstock genotype and Ψ_{leaf} suggests an effect of rootstock on the sensibility of grapevine to decreasing values Ψ_{leaf}, as hypothesized.

The significant interaction between scion and rootstock genotypes indicates an effect of rootstock on shifting the stomatal conductance towards higher or lower values in scions for a given Ψ_{leaf}. This effect was attributable to Merlot and Cabernet sauvignon for which significant differences in predicted g_s were observed between grapevines grafted on different rootstocks (Fig. 2). For both scions, there was a tendency for rootstocks possessing a *V. rupestris x V. berlandieri* (140Ru, 110R, 1103P) or a combined *V. rupestris x V. riparia* (101-14Mgt) genetic background to generate higher g_s values compared to rootstocks having a *V. riparia x V. berlandieri* (SO4, Teleki 5C) genetic background.

All methodological factors included in the analysis significantly explained a proportion of the variability observed in g_s values across the database (Table 1). The mean g_s predicted values for each methodological factor were compared by the model at a Ψ_{leaf} equal to -1.214 MPa corresponding to a moderate water stress (Ojeda 2008). Results show that values of g_s obtained from field studies (176.6 ± 3.7 mmol m^{-2} s^{-1}, Fig. 3A) were more than twice the one obtained in pot study settings (79.8 ± 3.7 mmol s^{-1} m^{-2}, $P < 0.0001$) in a moderate water stress situation. Also the mean g_s value obtained using a porometer (173 ± 5.6 mmol m^{-2} s^{-1}) was also more than twice the one obtained using IRGA (81.4 ± 1.9 mmol m^{-2} s^{-1}, $P < 0.0001$). These differences were clearly observable on scatterplots depicting the relationship between Ψ_{leaf} and predicted g_s (Fig. 3B, C, D) as well as in original data scatterplots (not shown). The mean predicted g_s value obtained along a seasonal time frame (134.7 ± 3.8 mmol m^{-2} s^{-1}) was significantly greater than the one obtained on a daily basis (110.2 ± 3.3 mmol s^{-1} m^{-2}, $P < 0.05$) although the proportion of the variability explained by this latter factor is minimizable (0.8%, Table 1). The final model equation (Equation 4) and the parameter coefficients allowing calculation of g_s according to this model are reported in Table S3.

The GLM allowed generating a classification of scions (Table 2) and rootstocks (Table 3) according to their mean predicted g_s in response to moderate water stress (model-determined mean Ψ_{leaf} reference equal to -1.214 MPa) independently of the interaction between scion and rootstock genotypes.

Thus, this classification allowed to significantly differentiate the archetypal near-anisohydric variety Syrah (predicted g_s: 160.86 ± 0.02 mmol m^{-2} s^{-1}) from the near-isohydric variety Grenache (predicted g_s: 67.51 ± 0.54 mmol m^{-2} s^{-1}) (Schultz 2003), for which the stomatal conductance was reduced more than twice compared to Syrah in similar water stress situation. As for rootstocks, the classification was dominated by *V. vinifera* genotypes in the lower mean g_s end of the table, whereas other *Vitis* crossings seemed to alternate despite the fact that the table clearly and significantly distinguished the recognized water stress "tolerant" rootstock 1103P (predicted g_s: 165.38 ± 0.03 mmol m^{-2} s^{-1}) (Bauerle et al. 2008) from the "sensitive" rootstock SO4 (89.07 ± 0.08 mmol m^{-2} s^{-1}) (Lovisolo et al. 2008b, Tramontini et al. 2013b).

Assessment of soil effect using a restricted Ψ_{leaf} database

The modelling performed on the database restricted to 3 scions explained 80.2% (r^2=0.802) of the variability in data distribution. This time neither scion nor rootstock genotypes were significant in predicting values of g_s (Table 4), while soil type ($P < 0.0001$, $\eta^2 = 19.2\%$) and method for g_s measurement ($P < 0.0001$, $\eta^2 = 43.2\%$) were significant, in addition to Ψ_{leaf} ($P < 0.0001$, $\eta^2 = 62.3\%$). For this database, the model compared the mean predicted g_s for each factor level at a Ψ_{leaf} reference equal to -1.144 MPa, corresponding to moderate water stress. Hence, similarly to the previous model, data obtained using a porometer (250.8 ± 7.6 mmol m^{-2} s^{-1}) were 3.6 times higher than using IRGA (69.2 ± 2.1 mmol m^{-2} s^{-1}) (Fig. 4A). Mean values of g_s predicted by the model were significantly higher in Intermediate soils (190.4 ± 4.7 mmol m^{-2} s^{-1}) than in water-Draining (141.7 ± 6.2 mmol m^{-2} s^{-1}) or –Retaining (104.7 ± 3.3 mmol m^{-2} s^{-1}) soils despite equal Ψ_{leaf} (Fig. 4B). There was a significant interaction between soil type (and thus water retention dynamics in the soil) and scion genotype indicating that transpiration rate varied with soil texture for a given scion, as depicted in Fig. 4C. Indeed a similar effect of soil water-holding properties was observed in Cabernet sauvignon and Syrah but not in Tempranillo for which water-Draining soil was associated with higher transpiration rates, followed by Intermediate soil and water-Retaining soil, which

was significantly different from the first. Of note, the higher values of g_s in Intermediate soils were not associated to porometer measurements as similar patterns of soil effect were observed in non-transformed g_s data obtained using IRGA only (not shown). The final model equation (Equation 5) and the parameter coefficients allowing calculation of g_s according to this model are reported in Table S4.

Discussion

A meta-analysis to reveal main factors regulating grapevine response to decreasing water availability

The results of this study confirm and extend previous findings suggesting that the genotype of scions and rootstocks, as well as soil water-holding properties influence water relations in grapevine. This study presented some shortcomings due to limitation in data availability: not every scion featured all rootstock genotypes or soil water-holding properties, and therefore separate analyses and grouping of data was necessary to assess some questions notably concerning the soil effect. In the same order of ideas some important environmental factors could not be assessed in this study simply because they were not reported in many of the studies used to collect data. One of them is vapour pressure deficit (VPD), which has been shown to modulate stomatal conductance in absence of changes in Ψ_{leaf} in well-watered grapevines (Perrone et al. 2012). This influence of VPD on g_s, thus independent of water stress, could moreover depend on scion genotype as suggested by studies in which near-isohydric varieties (i.e. Grenache) were shown to regulate stomatal aperture in response to increased VPD by synthesizing ABA locally in leaves whereas near-anisohydric varieties (i.e. Syrah) allowed Ψ_{leaf} to vary according to VPD without modifying stomatal aperture (Rogiers et al. 2012; Soar et al. 2006b). This phenomenon was qualified in a meta-analysis assessing iso- and anisohydric behaviours across plant species as sensitivity to VPD, uncovering that non-porous species (i.e. conifers) with low g_s had lower sensitivity to VPD compared to ring- or diffuse-porous species (Klein 2014). In addition to VPD, other factors known to influence water relations in grapevine such as shoot positioning (Lovisolo and Schubert 2000), age of grapevines (Düring 1994) and soil salinity (Prior et al. 1992) would also have enabled to push forward the scope of our analysis. Methodological details such as air temperature, CO_2 concentration and light intensity can also influence g_s; however, these factors were assumed to be standardized for the measurement of stomatal conductance (midday measurement on sunny days at atmospheric concentration of CO_2). Taken together these unconsidered factors may contribute to the unexplained proportion of variation in data distribution in the

model (< 20%). Lastly, it must also be acknowledged that the meta-analysis results are dependent on the content of the database and could differ if more or other studies were to be included.

Does rootstock affect scion iso- or anisohydric drought response, driving stomatal response to water deprivation?

The first model performed on the whole database enabled to test the hypothesis that scion and rootstock genotypes could influence water relations in grapevines (scion effect; rootstock effect and their interaction with Ψ_{leaf}) and that rootstocks can modify scions' response (interaction rootstock * scion). We could not at this stage group rootstocks according to their genetic background (i.e. *V. riparia* crossings *versus V. rupestris* crossings etc.) due to high intra-group variability related to the large number of scions inside each rootstock group. Nevertheless the first model could significantly distinguish Grenache and Syrah, the archetype near-isohydric and near-anisohydric varieties, independently of grafting status in response to moderate water stress (Table 2). Tolerance to water stress is associated to higher stomatal conductance, hydraulic conductance, photosynthesis rate and growth at lower soil water potential (reviewed by Bartlett et al. 2012). Here, at the far upper scale of stomatal aperture, the model significantly distinguished Muscat of Alexandria (212.44 ± 0.15 mmol m^{-2} s^{-1}), 110R (288.31 ± 0.07 mmol m^{-2} s^{-1}), Touriga (295.09 ± 0.012 mmol m^{-2} s^{-1}) and Sauvignon Blanc (338.17 ± 0.06 mmol m^{-2} s^{-1}). Sauvignon Blanc was not recognized as having an anisohydric behaviour in a study comparing different own-rooted varieties (Rogiers et al. 2009) but is commonly recognized as vigorous and was shown to have a rather low WUE (Tomás et al. 2014). In the present study, data representing Sauvignon blanc came from only one study though performed with a high number of replicate vines ($n = 18$-34) conferring considerable weight to this reference in the analysis (Naor, 1994). Of note, Ψ_{stem} data from the same field-grown grapevines grafted on a low vigour-inducing rootstock (216-3C) (Lovisolo et al. 2016) were published by the same author 3 years later (Naor et al. 1997) with a similar order of magnitude. As for Touriga and Muscat other references classified them as near-anisohydric varieties (Chaves et al. 2010, Lovisolo et al. 2010, Tomás et al. 2014) in accordance with their position in the present classification, in contrast to 110R

that was previously classified as isohydric (Lovisolo et al. 2010). The original data used in the present study for 110R show that depending on the degree of water stress, determined according to the level of stomatal closure, Ψ_{leaf} drops more ($g_s \approx 150$ mmol m^{-2} s^{-1}) or less ($g_s \approx 50$ mmol m^{-2} s^{-1}), resulting for this grapevine in a cloud of data without much linearity. This U-shaped curve could portray the activation of physiological mechanisms allowing recovery of Ψ_{leaf} at low g_s similar to those implicated in embolism recovery after rehydration and relying on an ABA flush to the leaves (Lovisolo et al. 2008a). Thus when data are put all together, such as in this model, the mean g_s for a Ψ_{leaf} corresponding to moderate stress (here Ψ_{leaf} = -1.214 MPa) is rather high, suggesting a near-anisohydric behaviour. Yet 110R is considered to be a vigour-inducing rootstock with high tolerance to water stress (Lovisolo et al. 2016) despite the fact that it was not significantly able to shift the Ψ_{leaf}-g_s curve in Cabernet sauvignon (see Figure 3), unlike other *V. rupestris × V. berlandieri* rootstocks (140Ru and 1103P).

When considered as scions, 110R significantly differed from 101-14Mgt (*V. riparia × V. rupestris*) at Ψ_{leaf} = -1.214 MPa in terms of stomatal aperture (Table 2). Interestingly, when considered as a rootstock (Table 3), 110R appeared in the middle range of stomatal apertures (123.7 ± 0.03 mmol m^{-2} s^{-1}) in response to moderate water stress close to 140Ru, and was not anymore significantly different from 101-14Mgt. This could represent the differential influence of leaf and root systems on the overall grapevine's response to decreasing Ψ_{leaf}. Grenache and Tempranillo were both classified with a very low tolerance to moderate water stress in both perspectives (as scion or rootstock) suggesting that both systems confer low tolerance through either hormonal or hydraulic mechanisms. Rootstock classification by the model also enabled to significantly differentiate between SO4 (*V. riparia × V. berlandieri*) and 1103P (*V. rupestris × V. berlandieri*), both of which significantly shifted the Ψ_{leaf}-g_s curve in Merlot and Cabernet Sauvignon in opposite direction (Fig. 2). SO4 was shown to have reduced hydraulic conductance in excised roots compared to 140Ru (*V. rupestris × V. berlandieri)* (Tramontini et al. 2013b) whereas longer root vessels, higher cross-sectional area of first order roots, larger trunk diameter and higher aquaporin expression were associated with improved root-system hydraulic conductance in 1103P compared to drought-sensitive rootstock 101-14Mgt (Alsina et al. 2011; Gambetta et al. 2012). Despite the significant

differences found for given rootstocks and scions, the data distribution suggests that a *continuum* exists in the range of stomatal sensitivity to water stress, rather than an isohydric - anisohydric dichotomy as stated more generally for plant species (Klein 2014). This *continuum* is further enriched by the diversity of scion-rootstock combinations and possibly by their interaction with soils with different water-holding properties.

Interference of methodological design, growth features and environment on biological behaviours

Data modelling also enabled to describe the effect of methodological factors related to study design. Thus grapevines studied in pot showed lower stomatal conductances compared to field-grown grapevines. This difference could be associated to the age of grapevines and the extent of root system development. Hence, the average age of grapevines studied in pots was 2.08 ± 1.88 years-old (range 0.5 to 8 years-old, 9/10 publications reported age) whereas for field studies, the average age was 9.45 ± 2.71 years-old (range 5-16, 31/37 publications reported age; $P < 0.001$, two-tailed unpaired t-test for unequal variance, not shown). The extent of the root system development could impact on signals transmitted to the scion, either hydraulic or hormonal, as young plants with reduced root surface are more susceptible to drought. Thus young plants could have an isohydric behaviour compared to older well-rooted plants and contribute to the intra-genotype variability in our analysis. Indeed it was shown that one-year-old Riesling vines (both ungrafted and grafted) show distinctly lower values of photosynthesis, stomatal conductance, carboxylation efficiency, and water use efficiency than their 6 years-old counterparts (Düring 1994). These rates were shown to increase over the years to reach equivalent values when vines are 4 years old. Interestingly, this effect was rootstock-dependent. Other underlying factors could contribute to the "pot" effect in reducing the stomatal conductance. Medrano and colleagues already discussed the fact that studies performed in greenhouse involves lower light exposure and faster water stress application compared to conditions found with field studies. They obtained comparable results to ours by showing reduced stomatal conductance values in function of decreasing predawn Ψ_{leaf} in potted vines (2 years-old Tempranillo) compared to field-grown vines (Medrano et al. 2002). This was accompanied by lower

electron transfer rates in potted grapevines as a function of predawn Ψ_{leaf} but similar rates when plotted against g_s. Bota and colleagues mentioned that it was more difficult to induce a slow and gradual water deficit in potted vines compared to field-grown vines that might be associated with altered extension of the root system. Nevertheless they were still able to show differences in stomatal regulation among rooted cuttings from different cultivars (Bota et al. 2001). Root system development is also dependant of rootstock genotype and soil properties (Alsina et al. 2011, Bauerle et al. 2008) further pointing out the complex interrelationship of the factors included in our model.

Higher values of stomatal conductances were associated with studies involving porometers (11 out of 40 studies). IRGA gradually replaced water conductance porometers over the years due to their higher reliability in terms of sensibility and accuracy. Closed system porometers are highly dependant on frequent calibration procedures and measurements are frequently biased by differences between leaf and atmospheric temperatures (Pearcy et al. 1989). The difference between measurement techniques is also obvious in untransformed g_s data (not shown) suggesting that there is indeed a real over estimation of stomatal conductance using porometers. In addition, a small but significant difference was observed between g_s values obtained over diurnal and seasonal cycles. One study suggested that changes in water relations occur along the vegetative cycle with higher stem Ψ (less negative) observed for similar stomatal conductances at pea-size berry compared to ripening, veraison and harvesting periods (Ciccarese et al. 2011). Nevertheless this factor contributed to explain only a small proportion of data variability (0.8%) and was not significant in the second analysis restricted to 3 scions. Overall, these findings stress the importance of considering methodological factors before comparing water relations in grapevines in-between studies.

Soil interference in controlling Ψ_{leaf}-g_s interrelationships have been statistically evidenced by using outputs of the restricted database. Interestingly, in soils with intermediate texture and not in water-retaining soils maximum stomatal function and consequent transpiration subsist. In addition, when results were split to the three varieties, maximum stomatal conductance in Tempranillo (an able stomatal controller of lowering water potential, Table 2) was even linked with water-draining soils. This further

underlines that water presence in the soil is not directly a promoter of grapevine transpiration, and that stress signals (possibly ABA) experienced in water-retaining soils and cultivar sensitivity to those signals have major control on stomatal closure (Tramontini et al. 2013a, Tramontini et al. 2014, Lovisolo et al. 2016).

Conclusions

To study Ψ_{leaf}-g_s interrelationships, we were able to include in a GLM biological (scion and rootstock genotypes), methodological (pot or field study, diurnal or seasonal monitoring of physiological parameters, and method for g_s assessment), and environmental (soil) factors. The model performed on the whole database explained 82.4% of the variation in data distribution with all factors explaining a significant proportion of the variability expressed, having the rootstock genotype the greatest contribution followed by the scion genotype. Importantly, the meta-analysis uncovered the effect of the method of measurement on values of g_s with consistently higher values in studies using a porometer compared to studies using IRGAs.

In accordance with our hypothesis, we found an effect of rootstock genotype on scion transpiration with *V. rupestris*-based rootstocks shifting the Ψ_{leaf}-g_s curve towards higher values of g_s in contrast to *V. riparia*-based rootstocks, showing a contrary effect. However this effect of rootstock on scion transpiration was surpassed by soil texture in the restricted database analysis, in which soil water-holding properties shifted the Ψ_{leaf}-g_s curve in a scion-dependant manner. According to different dynamics of water availability in soils where grapevines grew, scions showed generally higher stomatal conductance in studies conducted on soils with a loamy texture compared to the same scions studied on water-"draining" (sandy) or water-"retaining" (clayey) soils, despite displaying the same leaf water potential.

The GLM allowed generating a classification of scions and rootstocks according to their mean predicted g_s in response to moderate water stress (model-determined mean Ψ_{leaf} reference equal to -1.214 MPa), a value that we suggest to consider as statistically relevant to represent an average level of drought in grapevine cultivation.

In conclusion, we found a *continuum* range of grapevine stomatal sensitivities to water stress, rather than an isohydric- anisohydric dichotomy. This *continuum* is further enriched by scion/rootstock interaction with different soils. In future, experiments focused on drought responses in grapevine will be able to take advantage from this meta-analysis by comparing stomatal responses when leaf water potential is about -1.2 MPa, in order to reference (and possibly rank) the studied situation (plant, soil and experimental setup) with the statistical evidence here depicted from a literature survey.

Authors' contribution

ALL performed literature search, data acquisition and analysis, and contributed to the manuscript writing. DS contributed by designing the statistical analysis of data and reviewed the manuscript. PAR contributed to the manuscript through critical thinking, figure building and reviewing of the manuscript. CL contributed by designing the study and writing the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. References used in the meta-analysis

Table S2. Classification of soils

Table S3. Parameter coefficients allowing calculation of g_s according to the first model performed on the whole database

Table S4. Parameter coefficients allowing calculation of g_s according to the second model restricted to 3 scions performed to evaluate the effect of soil water-holding capacity

Table 1. Biological and methodological factors included in the first univariate general linear model performed on the whole database ($r^2 = 0.824$). ns: non significant; [a] non-significant factors were not included in the final model.

Factors	P-value	Partial η^2
Biological		
Scion genotype	< 0.0001	0.162
Rootstock genotype	< 0.0001	0.191
Methodological		
Pot or field study	< 0.0001	0.067
IRGA *vs* porometer	< 0.0001	0.086
Diurnal or seasonal	0.026	0.008
Covariate		
Ψ_{leaf}	< 0.0001	0.111
Interactions		
Scion genotype * Ψ_{leaf}	< 0.0001	0.145
Rootstock genotype * Ψ_{leaf}	< 0.0001	0.198
Scion genotype * Rootstock genotype	< 0.0001	0.116
Scion genotype * Rootstock genotype * Ψ_{leaf}	ns[a]	0.025

Table 2. Classification of scions according to their mean predicted stomatal conductance (g_s) in response to moderate water stress (leaf water potential, Ψ_{leaf} = -1.214 MPa).

Scion	Mean g_s (mmol m^{-2} s^{-1})	SE	95% confidence interval bounds		Significantly different (*P*<0.05) from
			Lower	Upper	
Carignan	44.97	0.60	15.38	95.06	Muscat of Alexandria, 110R, Sauvignon blanc, Touriga
Sylvaner	53.61	0.54	20.99	105.86	Muscat of Alexandria, 110R, Sauvignon blanc, Touriga
Tempranillo	60.48	0.33	30.17	104.16	Muscat of Alexandria, 110R, Sauvignon blanc, Touriga
Riesling	61.90	0.32	31.23	105.85	Muscat of Alexandria, 110R, Sauvignon blanc, Touriga
Grenache	67.51	0.06	49.41	89.12	Muscat of Alexandria, 110R, Sauvignon blanc, Syrah, Touriga
101-14Mgt	67.92	0.60	28.14	130.09	110R, Sauvignon blanc, Touriga
Manto negro	76.86	3.08	10.89	227.02	-
Athiri	79.11	0.29	44.11	126.96	Muscat of Alexandria, 110R, Sauvignon blanc, Touriga
Asyrtiko	86.17	0.32	47.95	138.52	110R, Sauvignon blanc, Touriga
Kekfrancos	98.99	0.91	40.35	191.29	Sauvignon blanc
Merlot	101.97	0.07	76.65	131.70	110R, Sauvignon blanc, Touriga
Portugieser	104.51	2.85	21.91	271.36	-
Sémillon	105.17	0.21	67.39	153.39	110R, Sauvignon blanc, Touriga
Malagouzia	106.07	0.14	73.49	146.03	110R, Sauvignon blanc, Touriga
Sangiovese	107.34	0.27	65.31	162.40	110R, Sauvignon blanc, Touriga
Aragones	122.76	0.20	81.89	173.89	110R, Sauvignon blanc, Touriga
Cabernet Sauvignon	124.74	0.03	104.72	146.95	110R, Sauvignon blanc, Touriga
Malvasia	129.36	1.06	55.46	243.31	-
Montepulciano	136.32	0.11	100.65	178.68	110R, Sauvignon blanc, Touriga
Trincadeira	157.20	0.29	102.40	226.65	Sauvignon blanc
Syrah	160.86	0.02	139.66	183.85	Grenache, 110R, Sauvignon blanc, Touriga
Chardonnay	187.65	0.45	115.86	281.04	-
Castelão	194.43	0.45	120.61	290.24	-
Muscat of Alexandria	212.44	0.15	160.19	273.83	Athiri, Carignan, Grenache, Riesling, Sylvaner, Tempranillo

Rosaki	269.76	1.65	128.93	476.61	-
110R	288.31	0.07	239.69	342.38	101-14Mgt, Aragones, Asyrtiko, Athiri, Cab. Sauvignon, Carignan, Grenache, Malagouzia, Merlot, Montepulciano, Riesling, Sangiovese, Sémillon, Sylvaner, Syrah, Tempranillo
Touriga	295.09	0.12	235.06	363.55	101-14Mgt, Aragones, Asyrtiko, Athiri, Cab. Sauvignon, Carignan, Grenache, Malagouzia, Merlot, Montepulciano, Riesling, Sangiovese, Sémillon, Sylvaner, Syrah, Tempranillo
Sauvignon blanc	338.17	0.06	288.35	392.78	101-14Mgt, Aragones, Asyrtiko, Athiri, Cab. Sauvignon, Carignan, Grenache, Kekfrancos, Malagouzia, Merlot, Montepulciano, Riesling, Sangiovese, Sémillon, Sylvaner, Syrah, Tempranillo, Trincadeira

Table 3. Classification of rootstocks according to their mean predicted stomatal conductance (g_s) in response to moderate water stress (leaf water potential, Ψ_{leaf} = -1.214 MPa).

Rootstock	Vitis crossing	Mean g_s (mmol m^{-2} s^{-1})	SE	95% confidence interval bounds		Significantly different ($P<0.05$) from
				Lower	Upper	
Grenache	*V. vinifera*	10.36	0.34	1.81	28.68	101-14Mgt, 1103P, 216-3C, 420A, Chardonnay, 3309C, K51-40, Ramsey, 110R, 99R, Ru140, Schawrzmann, Sémillon, SO4, Syrah, Teleki 5C
Tempranillo	*V. vinifera*	18.63	9.43	0.00	183.34	-
Manto Negro	*V. vinifera*	38.36	16.97	0.00	348.54	-
Carignan	*V. vinifera*	44.97	0.60	15.38	95.06	216-3C, 3309C
Sylvaner	*V. vinifera*	53.61	0.54	20.99	105.86	216-3C
Riesling	*V. vinifera*	61.90	0.32	31.23	105.85	216-3C, 3309C, Schawrzmann
161-49C	*V. riparia x V. berlandieri*	66.58	0.09	45.32	92.90	1103P, 216-3C, 3309C, K51-40, Schawrzmann, Teleki 5C
Kober 5BB	*V. riparia x V. berlandieri*	80.28	1.70	20.52	193.11	-
SO4	*V. riparia x V. berlandieri*	89.07	0.08	64.83	118.10	1103P, 216-3C, 3309C, Grenache, Schawrzmann
Sémillon	*V. vinifera*	105.17	0.21	67.39	153.39	216-3C, Grenache
140Ru	*V. rupestris x V. berlandieri*	122.67	0.02	103.74	143.52	216-3C, Grenache
99R	*V. rupestris x V. berlandieri*	122.76	0.20	81.89	173.89	216-3C, Grenache
110R	*V. rupestris x V. berlandieri*	123.66	0.03	104.02	145.33	216-3C, Grenache
Ramsey	*V. champinii*	128.49	0.06	101.12	159.82	216-3C, Grenache
Malvasia	*V. vinifera*	129.36	1.06	55.46	243.31	-
Cabernet Sauvignon	*V. vinifera*	132.11	1.14	55.45	251.32	-
420A	*V. riparia x V. berlandieri*	139.92	0.05	112.97	170.36	216-3C, Grenache
Syrah	*V. vinifera*	140.07	0.06	112.10	171.90	216-3C, Grenache
101-14Mgt	*V. rupestris x V. riparia*	142.41	0.07	111.83	177.57	216-3C, Grenache
Teleki 5C	*V. riparia x V. berlandieri*	158.76	0.03	134.31	185.71	161-49C, 216-3C, Grenache
K51-40	*V. rupestris x champinii*	161.50	0.07	128.43	199.19	161-49C, 216-3C, Grenache
1103 P	*V. rupestris x V. berlandieri*	165.38	0.03	142.10	190.85	161-49C, 216-3C, Grenache, SO4
Schawrzmann	*V. rupestris x V. riparia*	175.19	0.06	141.79	212.91	161-49C, 216-3C, Grenache, Riesling, SO4

3309 C	*V. rupestris x V. riparia*	196.50	0.11	151.21	249.10	161-49C, 216-3C, Carignan, Grenache, Riesling, SO4
Chardonnay	*V. vinifera*	231.82	1.60	106.17	419.86	Grenache
216-3C	*V. rupestris x V. solonis*	338.17	0.06	288.35	392.78	101-14Mgt, 1103P, 161-49C, 420A, Carignan, 3309C, Grenache, K51-40, Ramsey, 110R, 99R, Riesling, Sémillon, SO4, Sylvaner, Syrah, Teleki 5C

Table 4. Contribution of the biological, methodological and environmental factors in predicting stomatal conductance (g_s) values in the univariate general linear model performed on the database restricted to 3 scions (Cabernet sauvignon, Syrah and Tempranillo). ns: non significant; NA: Not applicable because of unbalanced distribution of data; [a] non-significant factors were not included in the final model.

Factors	*P*-value	Partial η^2
Biological factors		
Scion genotype	ns[a]	0.005
Rootstock genotype family	ns	0.016
Methodological factors		
IRGA *versus* porometer	<0.0001	0.432
Pot or field study	ns	ns
Diurnal or seasonal	ns	ns
Environmental factor		
Soil type	<0.0001	0.197
Covariate		
Ψ_{leaf}	<0.0001	0.623
Interactions		
Soil type * Scion genotype	0.001	0.135
Soil type * Rootstock genotype family	NA	0

Figure legends

Fig. 1. Data modelling using the univariate general linear model (GLM). (A) Original stomatal conductance (g_s) data plotted against leaf water potential (Ψ_{leaf}). Only data representing water-stressed grapevines ($\Psi_{leaf} < -0.5$) are represented and follow a logarithmic distribution. (B) Data linearized using a BOX COX transformation of g_s using the power of 0.4. The GLM was run on transformed data and the normality of predicted residuals was tested using both statistical and visual analysis. The Normal Q-Q plot (C) shows normally distributed residual data after removing outlier values. (D) Predicted distribution of data according to the GLM showing varying vertical $\Psi_{leaf} - g_s$ curve distribution (transpiration activity) and slopes (stomatal sensibility to water stress) across different scions.

Fig. 2. Differences in predicted g_s within Merlot and Cabernet sauvignon cultivars grafted on different rootstocks. For each scion, the model attributed a reference rootstock (value = 0; Merlot grafted on SO4 and Cabernet sauvignon grafted on Teleki C) and calculated the differences in predicted g_s values with the other rootstocks. The genetic background of the rootstocks are as follow: *V. rupestris x V. berlandieri* for 140Ru, 110R and 1103P; *V. rupestris x V. riparia* for 101-14Mgt; *V. riparia x V. berlandieri* for SO4 and Teleki 5C; *V. champinii* for Ramsey; and *V. vinifera* for Cabernet Sauvignon. Bars indicate mean and SEM. * indicates that the difference from the reference is significant at $P < 0.05$.

Fig. 3. Influence of methodological factors in predicting g_s values. (A) Mean predicted g_s values in grapevines according to the different methodological factors assessed in the GLM. All levels of methodological factors were compared by the model at $\Psi_{leaf} = -1.214$ MPa, corresponding to moderate water stress. The number of references and data associated to each level is indicated above each bar (*n* references; *n* data). Scatterplots depicting predicted g_s against Ψ_{leaf} according to each methodological factors are shown in (B) potted *versus* field-grown grapevines; (C) method used for measuring g_s; and (D)

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time frame for measurements. Bars indicate mean and SEM. * ($P < 0.05$) and *** ($P < 0.0001$) indicate significant differences between means.

Fig. 4. Influence of methodological and environmental factors on predicted g_s values in the analysis restricted to Cabernet sauvignon, Syrah and Tempranillo. (A) Predicted values of g_s in studies using IRGA and porometer. (B) Predicted values of g_s in water-Draining, Intermediate and water-Retaining soils. (C) Interaction of scion genotype with soil water-holding properties in predicting g_s values: white bars represent water-Draining, black Intermediate, and gray water-Retaining soils. Mean predicted values of g_s for all factor levels were compared at Ψ_{leaf} = - 1.144 MPa, corresponding to moderate water stress. The number of references and data associated to each level is indicated above each bar (*n* references; *n* data). Bars indicate mean and SEM. * ($P < 0.05$) and *** ($P < 0.0001$) indicate significant differences between means.
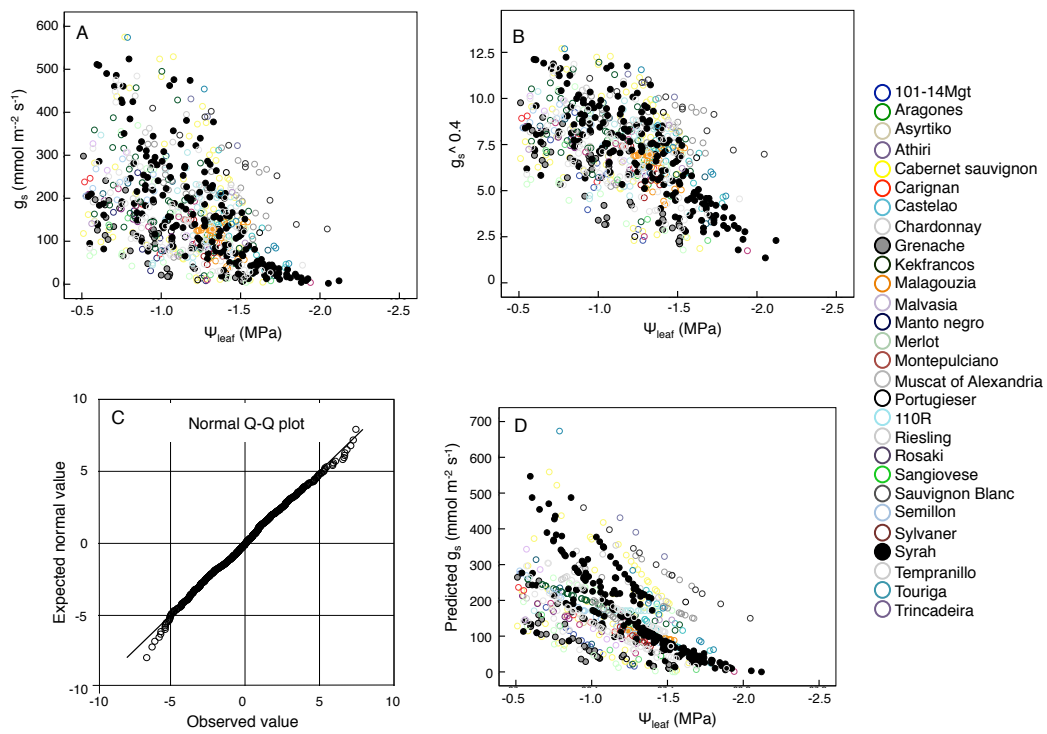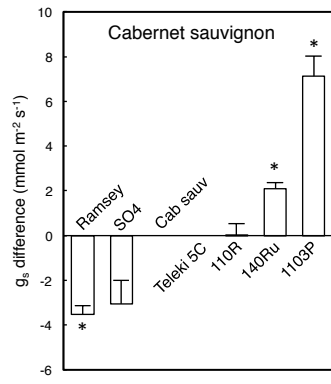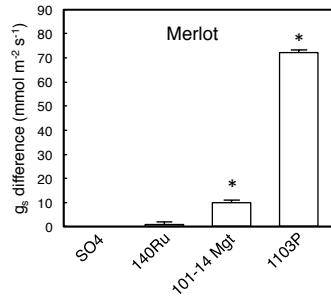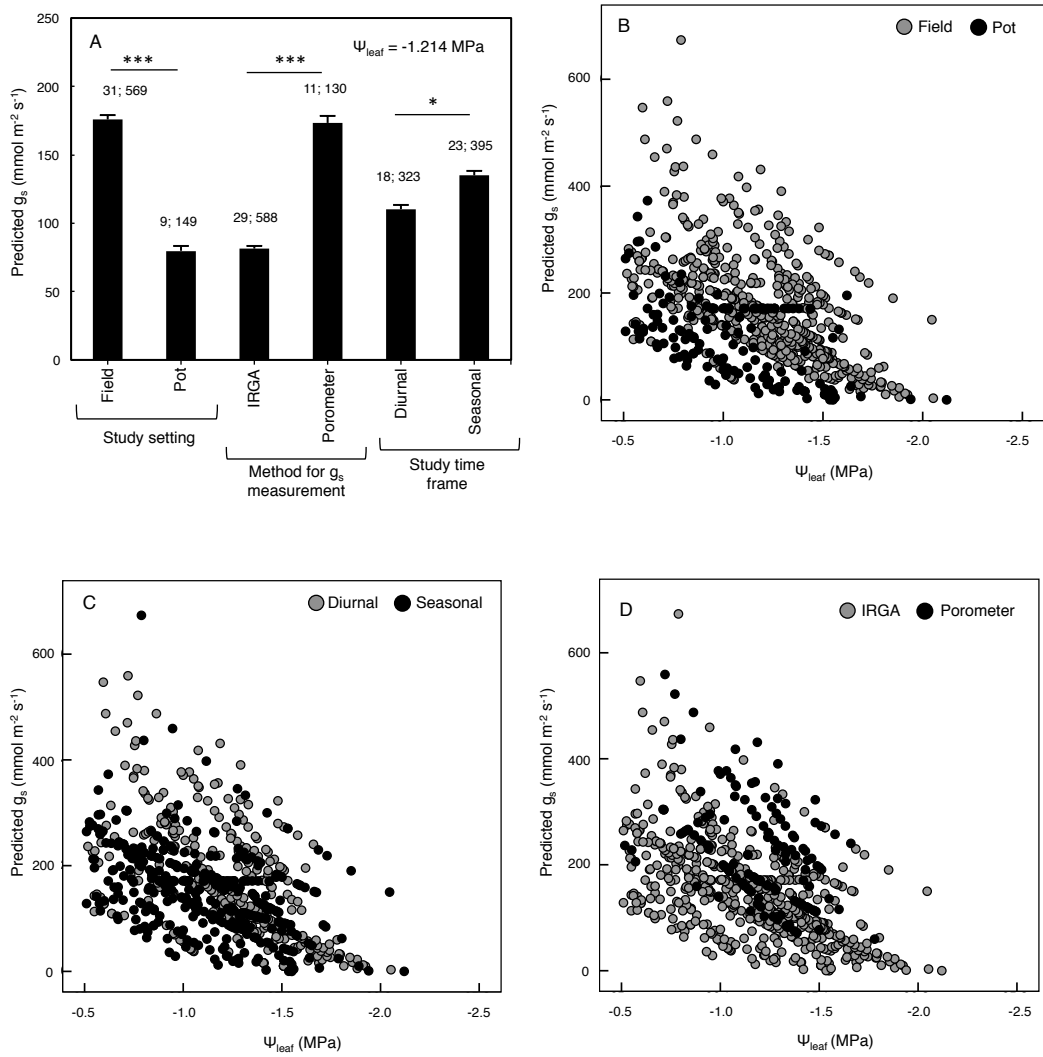
Figure 1

Figure 2

Figure 3

Figure 4