Exploring the genetic variability in water use efficiency: evaluation of inter and intra cultivar genetic diversity in grapevines

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Highlights:

- The WUE_i can be used as selection criteria to improve WUE in grapevines
- There is a wide variability of the WUE_i among grapevine cultivars
- Inside clones of Tempranillo the WUEi variability is around 80% than among cultivars
- Some cultivars and clones with higher and lower WUE_i than the average were identified

Abstract

Genetic improvement of crop Water Use Efficiency (WUE) is a general goal because the increasing water scarcity and the trend to a more sustainable agriculture. For grapevines, this subject is relevant and need an urgent response because their wide distribution in semi-arid areas. New cultivars are difficult to introduce in viticulture due to the narrow dependency of consumer appreciation often linked to a certain particular wine taste. Clones of reputed cultivars would presumably be more accepted but little is known on the intra-cultivar genetic variability of the WUE. The present work compares, on the basis of two field assays, the variability of intrinsic water use efficiency (WUE_i) in a large collection of cultivars in contrast with a collection of clones of Tempranillo cultivar. The results show that clonal variability of WUE_i was around 80% of the inter-cultivar, thus providing a first assessment on the opportunity for clonal selection by WUE. Plotting the WUE_i data against stem water potential or stomatal conductance it was possible to identify cultivars and clones out of the confidence intervals of this linear regression thus with significantly higher and lower WUE_i values. The present results contribute to open the expectative for a genetic improvement of grapevine WUE

Key words: *Vitis vinifera*, water stress, clone, genotype evaluation, Tempranillo.

1. Introduction

Water use efficiency issue is becoming an important subject for environmental sustainability of crops because of growing necessity of food production and increasing needs of irrigation to enhance crop productivity [1–3].

Viticulture is mainly located in semi-arid areas [3,4]. High water requirements are common because the growth cycle of grapevines coincides with the driest months, making irrigation a convenient practice. That means an important consume of water which sometimes enter into conflict with the economic and environmental viability of this crop, particularly in dry prone areas. Water use by irrigation is therefore limiting the sustainability of the vineyards and sometimes also enters in competition with other critical human uses [5–7]. Stressing even more the conflict, the climate change forecasts and last year's climatic data show increasing drought and heat waves frequency joined with higher unpredictability of rainfall [8,9]. According to climate change predictions, episodes of extreme drought will be from 3 to 8 times more frequent than at the present [10]. It means that, for most of the Mediterranean and semi-arid regions of grapevine production, high irrigation volumes will be required to obtain a reasonable harvest.

In consequence, the optimization of water use for vineyards, by improving water use efficiency (WUE), is one of the highest interest subjects. As a response to this social claim, an important volume of applied and fundamental research has been focused into the exploration of the capacity to optimize grapevine water use. Such optimization is explored directly adjusting the irrigation timing and schedule introducing new technologies to reduce water consumption [11–14] looking for a compromise between reasonable crop yield and quality and reduction of irrigation dosage.

However, the successful results of genetic selection and the advances in genomics and phenotyping [15,16] also impulse to exploit the *Vitis* genetic diversity to enhance the stress resistance and the improvement of WUE[17]. The impressive world-wide amount of grapevine cultivars used in viticulture and already properly described [18,19] provides wide genetic variability of the grapevine genome which constitute invaluable genetic resource to reach with crop adaptation to the different environmental conditions and potentially to climate change.

Since, little is known about the WUE as a selection criteria in grapevines, even though some cultivars are reputed as more adapted to drought-prone conditions, thus, presumably showing, in some way higher WUE.

Genetic variability in WUE in grapevines was early demonstrated in our group [20] measuring intrinsic leaf water use efficiency (WUE_i) as the quotient of photosynthesis rate versus stomatal conductance (A_N/g_s) in a collection of old Majorcan cultivars, and by Gaudillere et al. [21], estimating WUE as the surrogate character isotope ^{13}C discrimination in biomass ($\delta^{13}C$). After these early works, significant differences in different physiological parameters closely related to the WUE such as A_N and g_s as well as WUE_i were published by Souza et al. [22]; Soar et al. [23]; Pou et al. [24]; Prieto et al. [25]; Costa et al. [26];Tomás et al. [27]; Tomás et al. [28]; Bota et al. [29] showing interesting variability in WUE_i, in leaf or grape $\delta^{13}C$, or whole plant WUE among different cultivars. A wide range of A_N/g_s among genotypes under high soil water availability and soil water stress conditions was demonstrated also for field growing plants [29].

However, it has been shown that within a single plant, there is a large variation of A_N/g_s clearly dependent on prevailing environmental conditions [30,31]. The high environmentally-induced variation on intercepted light, and other parameters was always present limiting the capacity to evaluate the genetic variability among cultivars. Moreover, most of the measurements of WUE in grapevines were performed at the leaf level and the comparisons with the measurements at whole-plant level showed to be sometimes good but poor in other experiments [3,27,28]. The lack of correlation was related to the complexity of the grapevines canopy structure [32], night transpiration [33], and the important role of plant respiration [34,35]. Nevertheless, the necessity to evaluate the WUE genetic variation previous to any breeding program obligates to explore it on the basis of single, rapid measurements parameters (as A_N/gs) as selection criteria.

The end goals of those genetic variability assessments were also to identify some cultivar with higher WUE which could help to reduce the watering necessities thus improving the WUE at the vineyards. Furthermore, for commercial viticulture, the agricultural behavior, the fruit quality characteristics and particular wine taste of a certain cultivar are crucial for the final wine production. Therefore to change current cultivars for others with higher WUE would be matter of wide discussions likely reducing the applicability of new obtentions. In such a way, there is a large tradition of clonal selection inside a certain cultivar which was matter of wide research for more adjusted productivity, higher diseases resistance or particular adaptation to limiting environmental characteristics [36–38]. Different grapevine clonal selection programs were already made at the regional level for public research as for private nurseries with reasonable successful results [39,40]. Particularly, inside the Tempranillo, a Spanish cultivar widespread in Spain and around the world, a wide effort was done in different Spanish regions to select clones better adapted to different characteristics, with results recently analyzed by Ibáñez et al. [39].

In the light of these results , it seems interesting to look for the variability of the WUE among clone collections already shown as a source of important variability for agronomic and quality characters in grapevine [38].

Tempranillo cultivar has also been mater of wide studies on its agronomic behavior, physiological performance and genetic background [39,41], and moreover there is a large collection of Tempranillo commercial clones available at public research and nurseries. The wide differentiation among grapevine clones in productivity, quality and other complex characters reinforces the interest to explore such variability for the WUE parameters.

On these grounds, the present work shows a particular insight on the variability of the WUE among different cultivars as well as among different clones of Tempranillo cultivar. The results open a new field of research for high-WUE addressed selection inside elite cultivars of grapevine.

2. Material and Methods:

2.1 Plant material

Twenty three grapevine cultivars (Table 1) were studied during August 2011 in an experimental vineyard located in Palma de Mallorca (39º 35´N, 2º39´E) (Balearic Islands, Spain). Plants were 12 year old, grafted onto 99-Richter rootstock, trained as doubled cordon and similarly pruned and managed on a standard procedure. These cultivars include 16 Majorcan local and seven widely cultivated around the world. These grapevine cultivars include Tempranillo, clone RJ43, the widest distributed clone of this cultivar in Spain. Soil presented a loamy texture with alkaline pH due to high concentrations of active limestone and carbonates. Plot was generously irrigated until flowering remaining rain fed until harvest.

Furthermore, 30 Tempranillo genotypes (Table 1) including commercial clones selected by agronomic characters, and different accessions chosen by life cycle duration were measured in Logroño (La Rioja, Spain) and its surroundings (42º 28´ N, 2º 27’ W). Data acquisition of those clones was done by August 2015. These clones were selected by three different entities and studied on their own experimental field: one public institute (ICVV – Instituto de las Ciencias de la Vid y el Vino) and two commercial wine nurseries (Viveros Provedo S.A. and Vitis Navarra Selección SA). The key name used here for each genotype represents the institute where clones were selected (i.e. a number for ICVV accessions; VP for Viveros Provedo and VN for Vitis Navarra). The clones collection includes the most commercial Tempranillo ones (especially RJ43). The RJ26, RJ43, RJ51 and RJ78 were coincident in different locations. The soils of the three experimental fields (VP, VN and ICVV) presented a clay texture and rested upon limestone and carbonates, as is typical on La Rioja.

All clones were grafted onto 110-Richter rootstock, trained as doubled cordon and similarly pruned and managed on a standard procedure. Because watering was not identical among locations, the data was always referred to the particular plant water status estimated as water potential or stomatal conductance.

The two rootstocks used in both experiments are so close genetically, that the influence on the plant behavior results negligible. Because this work was focused on the evaluation of the genetic variability between the cultivars themselves and between clones themselves both independent experiments were considered.

2.2 Plant water status

The plant water status was estimated by midday stem water potential (Ψ_{stem}) measured with a Scholander pressure chamber (Soil moisture Equipment Corp. Santa Barbara, California USA). Ψ_{stem} was measured at midday (between 13:00 and 14:00 h, local time) on non-transpiring leaves that had been bagged with both plastic sheet and aluminum foil at least 1 h before measurement. Bagging prevented leaf transpiration, so leaf water potential equaled stem water potential [42]. Ψ_{stem} was measured on one leaf per plant in three to six plants for genotype

2.3 Gas exchange measurements

Leaf net photosynthesis (A_N) and stomatal conductance (g_s) were measured in fully exposed mature leaf (one per plant, n=3-6) in the same day of plant water status measurements. All determinations were done between 10:00 and 13:00 h (local time) using an infrared open gas exchange analyzer system (Li-6400, Li-cor Inc., Lincoln, Nebraska, USA) in both studies. The CO_2 concentration inside the chamber was 400 µmol CO_2 mol^{-1} air, PAR was always above saturation levels and temperature ranged between 30 and 33ºC. Intrinsic water use efficiency (WUE_i) was calculated as the ratio between leaf net photosynthesis (A_N) and stomatal conductance (g_s). As the check on the measurement time effect showed not clear trend, this parameter is little affected by environmental conditions, in contrast with the similar check for instantaneous WUE (A_n/E). (See Supplementary Figure 1)

2.4 Statistical analysis

Regression coefficients, correlations and box plots were obtained using 12.0 Sigmaplot software package (SPSS; Chicago, IL, USA). The coefficient of variation (CV) is defined as the ratio of the standard deviation divided by the average. The normality of the two data sets was verified.

3. Results

3.1 Climatic conditions

Climatic conditions of the two locations were typical of Mediterranean climate, with high temperatures and irradiance during summer (Table 2). However, slight differences were observed between experiments. In Palma de Mallorca assay, mean temperature of June, July and August was higher than in Logroño (2-3 ºC difference), but, on the other hand, irradiance was lower. Nevertheless, reference evapotranspiration values were very similar in both locations for the three months of experiments. A certain difference in total rainfall was present between locations, with very low amount of rain in Palma de Mallorca, and moderate precipitation in Logroño during this summer (Table 2). Despite these differences, the plant water status was very similar in both locations (see the next section) because of a week's period from the last rains to the measurement dates in Logroño. However, for the clone's measurements there were clear differences among Logroño locations in terms of soil water availability which was evident analyzing Ψ_{stem} and g_s values. Because of those differences, the results obtained in these experiments has been arranged in three categories on the basis of g_s values, according to Medrano et al. [43] and Flexas et al. [44] : plants under non water stress conditions (g_s>0.150 mol H_2O $m^{-2}s^{-1}$), moderate water stress (g_s between 0.150-0.075 mol H_2O $m^{-2}s^{-1}$) and severe water stress (g_s<0.075 mol H_2O $m^{-2}s^{-1}$). Even though the g_s max was not available for clone comparisons, the study of inter-cultivars data set, enabled to compare for this experiment gs max, and gs reduction by drought. A strong correlation was evident between decrease of g_s (as percentage) and gs measured under moderate and severe water stress (r^2= 0.88, p-value< 0.0001; Supplementary Figure 2).

3.2 Genotype performance and water stress

Figures 1 and 2 shows the average values of stomatal conductance (g_s) and intrinsic water use efficiency (WUE_i) of the cultivars (Figure 1) clones and accessions of Tempranillo cultivar (Figure 2). The wide range variation of g_s observed between cultivars (Fig 1A), evidenced the different behavior for similar water availability conditions. As expected, the cultivars showing lower g_s values, as Giró Ros, Callet Blanc, Gorgollasa, Moll and Vinater blanc, presented higher values of WUE_i (Fig 1B). In contrast, Malvasía, Macabeo, Escursac and Chardonnay showed the highest g_s values and in parallel, the lowest WUE_i.

Inside cultivar plots, Tempranillo remained in an intermediate position. This cultivar, under moderate water stress conditions showed a WUE_i around 100 μmol CO_2 mol H_2O^{-1} at the Majorca experiment (Fig 1B). Regarding Tempranillo cv. clones and accessions, the average WUE_i values were a little higher and interestingly, inside this group of moderate water stress, the values ranged from 80 to 136 μmol CO_2 mol H_2O^{-1} (Fig 2B). This range of variation among clones resulted similar to those found among cultivars in the Majorca experiment (118-70 μmol CO_2 mol H_2O^{-1}).

It is highlighted that, for severe water stress, the WUE_i values were higher than for moderate and non-stressed conditions, achieving 120 μmol CO_2 mol H_2O^{-1} for local cultivars in Majorca experiment as Callet (Fig 1B). Inside Tempranillo clones the results showed a WUE_i average of 130 μmol CO_2 mol H_2O^{1} with the highest values for 807 accession achieving 147 μmol CO_2 mol H_2O^{-1} (Fig 2B).

Because not all cultivars and clones are inside the moderate water stress range, the WUE_i results were also compared in reference to soil water availability conditions. In order to evaluate the particular WUE_i of each genotype over the general regression line of WUE_i plotted against the soil water availability stablished as stem water potential (Ψ_{stem}) (Fig 3A) or stomatal conductance (g_s) (Fig 3B). The plots of figures 3 and 4 show the regression line for cultivars and clones respectively, as well as the lines corresponding to confidence intervals of 95%. In that way, genotypes out of this band can be qualified as higher/lower WUE_i than average values. Interestingly, those regression lines showed a regression coefficient which was clearly higher for clones than for cultivars. As showed in Figure 3, the dependence of WUE_i was lower against Ψ_{stem} than against g_s, being statistically significant only against g_s. However, for the Tempranillo clones (Figure 4), both regression lines showed to be statistically significant. These plots enable to identify some outside cultivars with higher (Callet, Esperó de gall, Gallmeter Valent Blanc) and lower (Argamussa, Shiraz, Vinater Blanc and Malvasía) WUE_i (Figure 3) than the average. For clones, the ones with better WUE_i

than expected were 814, 260, 280, 160, RJ26 and 518, and with a lower WUE_i than expected VN1, RJ51 and 1041(Figure 4).

These differences between cultivars and clones in g_s and WUE_i average values are more clearly showed in table 3 and table 4 as well as the predicted value calculated from figure 3 and 4 correspondences. The relative differences between the predicted value of WUE_i and the measured one are also shown. Higher (positive and negative) values of this ratio can be used to identify the extreme genotypes in terms of WUE_i. For example, under severe water stress, Callet Blanc and Vinater Blanc showed the extreme ratio that was near to 18% higher and lower respectively (Table 3). Under moderate water stress conditions, five cultivars showed higher ratio, being Tempranillo one of these cultivars. In case of Tempranillo clones, it is remarkable RJ26 that showed in both non stressed and moderate water stress conditions an 18% and 9 % higher WUEi ratios respectively (Table 4). Interestingly, those genotypes also showed an important variability of WUE_i.

3.3 Genetic variability of WUE

Coefficient of variance is commonly reputed as good estimator of variability for different characters. Table 5 shows the standard deviation and coefficient of variance (CV) for g_s and WUE_i among the cultivars and clones. The CV values showed to be lower between clones than between cultivars in both g_s and WUE_i parameters, achieving for clones a 70-80% of the cultivars variance. Thus, the restriction to a single cultivar clones affected the range of variation of the WUE_i. Nevertheless, it remains in considerable high values which argues in favour at a possible use of this variation for clonal selection purposes.

4. Discussion

The improvement of crop water use efficiency is a general goal widely claimed and inside this, the genetic improvement seems a promising field of research. Mainly on the grounds of the known progress achieved with elite cultivars in different crops. For grapevines, such WUE improvement results even more urgent to secure the sustainability of this crop in high quality reputed areas [3,5]. Recent results showed by different ways the existence of considerable genetic variability among cultivars of grapevine [3,4]. However, as stated above, in contrast with other crops, to change the stablished grapevine cultivars in a determined region is difficult because of the narrow dependency of consumer appreciation often linked to a certain particular wine taste.

In that way, the present work was aimed to explore the genetic diversity among clones of a certain cultivar, showing by first time that there is considerable genetic variability among clones of Tempranillo cultivar. These data are promising to improve the genetic selection of the grapevine WUE, and also an interesting insight on the plasticity of the plants genome to generate variability linked to asexual reproduction maintaining cultivar phenotypic characteristics.

The field growth assay concerning different cultivars shows (Figure 1) that inter-cultivar variations of WUE are on a similar range to the previously reported in pots and field experiments in our group under Mediterranean conditions [20,27,28] and other groups in field and pot experiments [21,25,45,46]. The present results show that Tempranillo WUE_i values were, under moderate water stress conditions close to the general average, as reported in previous pot and field experiments [27,28,35].

Under similar environmental conditions and under a moderate water stress, the Tempranillo clones showed a range of variation of WUE_i (80 to 136 μmol CO_2mol H_2O^{-1}) which was lower but close to the found for the inter cultivars comparison (69 to 118 μmol CO_2mol H_2O^{-1}) (Figures 1 and 2). This interesting result shows that for WUE_i there is a wide intra-varietal variability which could be exploited to identify elite genotypes. The previous work on clonal selection by different agronomic characters also showed the existence of an impressive genetic variation among clones in grapevine. Interesting variability for WUE was already published for potato [47] coffee [48], and poplar clones [49]. Even though is not matter of this work to identify the genetic basis of such variability, it seems now affordable to do it in near future.

Moreover, the analysis of the WUE_i correspondence with Ψ_{stem} or g_s (Figure 3) showed that it is possible to identify some genotypes out of the 95% confidence interval with higher and lower WUE_i than the average at any water availability condition. Such analysis showed more significant results for clones than for cultivars. Any case, this analysis enables to identify cultivars and clones with WUE_i significantly different than the average. The higher correspondence of clones plot could be understood as a result of the higher genetic homogeneity among them. This reinforces the reliability of this analysis to identify genotypes performing out of the average.

The identification of these genotypes and the contrast between expected and actual values (Table 4) provides an early identification and evaluation of such cultivars and clones by the difference between observed and predicted values. Interestingly, for identified cultivars, WUE_i ranged among a 23% higher than expected (Callet) and 17% lower than expected (Vinater Blanc), both cultivars original from Majorca. Regarding the clones, the range of WUE_i was shorter (+17%, (814) and -16% (RJ51)), but still quite high to be confident to have the ability to success with a new specific selection programs. This was also confirmed comparing the coefficient of the variance of the WUE_i data for cultivars and clones. The CV value for clones was around 80% of the cultivars, thus confirming that even though the variability is lower, but it is still enough.

This confirms the important source of genetic variability inside the cultivar which could be exploited. As it was the case in previous clonal selection programs favoring certain plant morphological characteristics [41] phenological cycle [50]; plant yield[51], fungi resistance [52], or specific fruit quality parameters [38]. Those successful selection programs also relays on the high genetic diversity of this specie early shown by different ways [53]. As well these results are in accordance with recent reports about inter and intra varietal genetic diversity of this specie [37,54] showing that somatic mutations accumulation is a powerful way to generate genetic variability in plants [55,56].

Along the last 30 years, a lot of different clonal selection programs were applied to the Tempranillo cultivar focused on the improvement of different agronomic characteristics providing specific clones adapted to a particular soil or climatic conditions [39]. On the grounds of the present results, the expectative for a genetic improvement of grapevine WUE seems to be closer and realistic.

However, the scarcity of successful results in other crops selecting for this character, as well as the known difficulties when scaling up from leaf to whole plant WUE [3], advise to be cautious in reaching these results. A wider effort will be needed to find a way for the genetic improvement of WUE.

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6. References

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Figure Captions

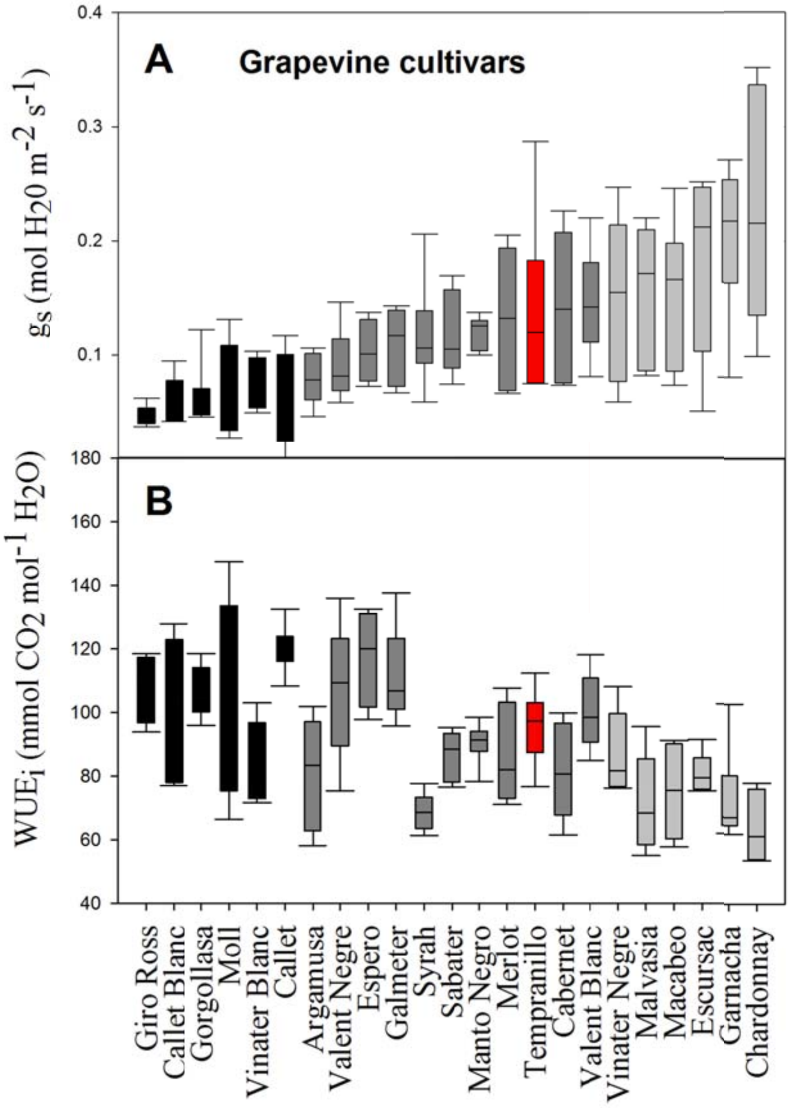

Figure1. Box plot for stomatal conductance (g_s) (A) and intrinsic water use efficiency (WUEi) (B) for each grapevine cultivar measured during the experiment. The horizontal lines that intersect the box are the medians. The lines that delimit the bottom and the top of the boxes are the 25th and 75th percentiles, respectively. The 10th and 90th percentiles are indicated by whiskers. The extreme values are represented as the dots (outliers). Black fill: severe water stress group, dark grey fill; moderate water-stress and grey fill non water-stressed group. Tempranillo cv. appeared as red filled in the moderate water-stress group.

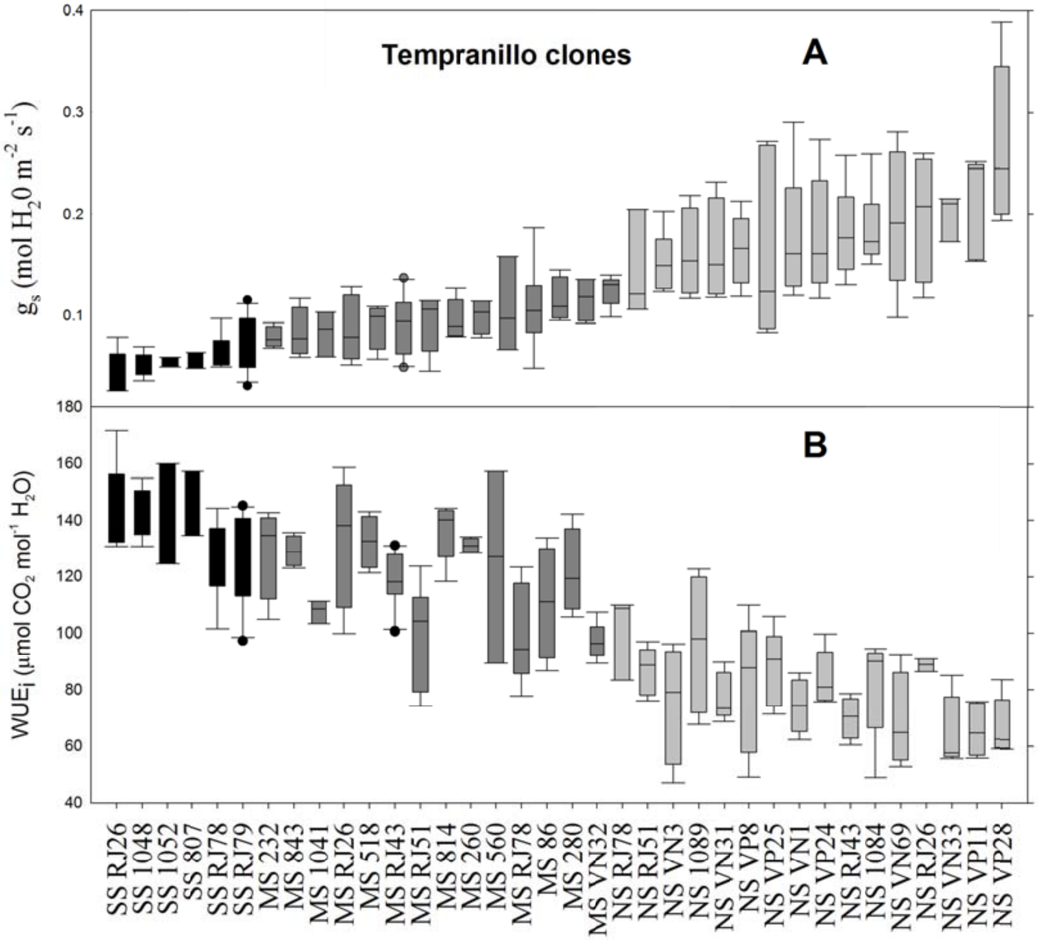

Figure 2. Box plot for stomatal conductance (g_s) (A) and intrinsic water use efficiency (WUEi) (B) for each grapevine Tempranillo clone measured during the experiment. The horizontal lines that intersect the box are the medians. The lines that delimit the bottom and the top of the boxes are the 25th and 75th percentiles, respectively. The 10th and 90th percentiles are indicated by whiskers. The extreme values are represented as the dots (outliers). Black fill: severe water stress group, dark grey fill; moderate water-stress and grey fill non water-stressed group. Tempranillo cv. appeared as red filled in the moderate water-stress group.

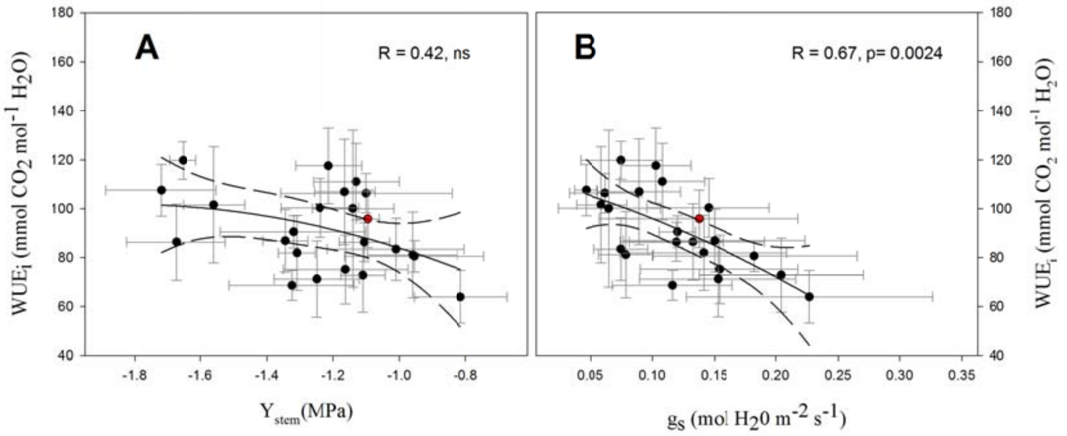

Figure 3. Correlations between WUEi and stem water potential (Ψ_{stem}) (A) and stomatal conductance (g_s) (B) for grapevine cultivars. Data are mean (n=6) ± standard error. Dashed lines showed confidence interval (95%).

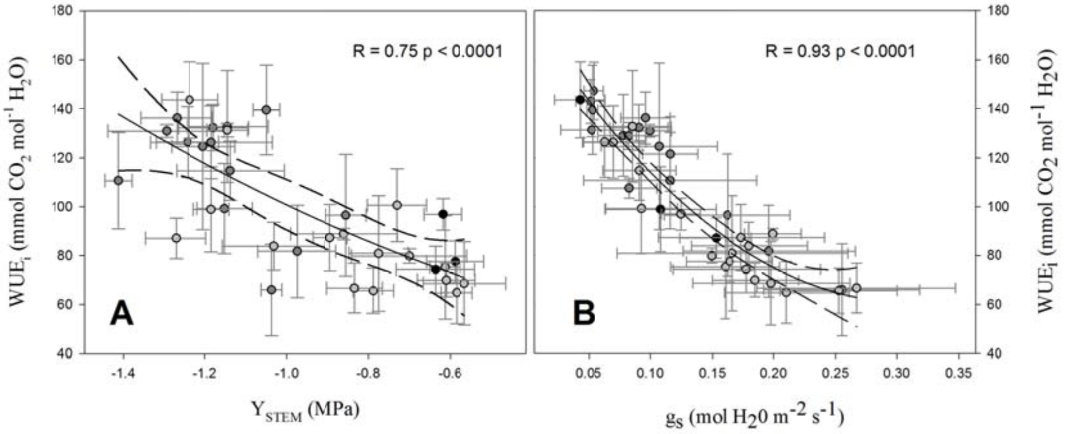

Figure 4. Correlations between WUEi and stem water potential (Ψ_{stem}) (A) and stomatal conductance (g_s) (B) of Tempranillo clones. Data are means (n=3-6) ±standard error. Dashed lines show confidence interval (95%).

Table 1. List of plant material

Grapevine cultivars

Berry color	Cultivar
Red	Cabernet Sauvignon
	Callet
	Escursac
	Esperó de Gall
	Galmeter
	Garnacha
	Gorgollasa
	Manto Negro
	Merlot
	Sabater
	Syrah
	Tempranillo
	Valent Negre
	Vinater Negre
White	Argamusa
	Callet Blanc
	Chardonnay
	Girrdonn
	Macabeo
	Malvasia de Banyalbufar
	Moll
	Valent Blanc
	Vinater Blanc

Tempranillo clones

	Name	Plot
Accession	86	+
	232	+
	260	+
	280	+
	518	+
	560	+
	807	+
	814	+
	843	+
	1041	+
	1048	+
	1052	+
	1084	+
	1089	+
Selection	RJ26	+++
	RJ43	+++
	RJ51	++
	RJ78	+++
	RJ79	+
	VN1	+
	VN3	+
	VN31	+
	VN32	+
	VN33	+
	VN69	+
	VP8	+
	VP11	+
	VP24	+
	VP25	+
	VP28	+

Table 2. Monthly mean temperature, monthly accumulative rainfall, reference evapotranspiration (ETo) and irradiance during summer time of 2011 in Palma de Mallorca and summer 2015 in Logroño.

Location	Month	Mean Tª (ºC)	Rainfall (mm)	ETo (mm)	Irradiance (Mj m^{-2} day^{-1})
Palma de Mallorca	June	21.52 ± 0.55	25.8	149.67	24.88 ± 1.09
	July	24.40 ± 0.28	17.2	160.86	23.81 ± 0.91
	August	25.83 ± 0.28	0	156.45	23.23 ± 0.44
Logroño	June	19.80 ± 3.30	82.6	145.7	25.62 ± 6.33
	July	22.53 ± 2.47	42.4	155.2	25.40 ± 4.94
	August	21.10 ± 2.77	13.2	127.7	21.60 ± 4.90

Table 3. Values of g_s, actual WUE_i, predicted WUE_i (calculated from figure 3B) and ratio between actual and predicted WUE_i of 12 grapevine cultivars. Cultivars showed were those out of confidence interval (95%). Data are mean ± SD (n=6).

Group	Cultivar	g_s (mol m^{-2} s^{-1})	WUE_i (μmol CO_2 mol^{-1} H_2O)	Predicted WUE_i (μmol CO_2 mol^{-1} H_2O)	Predicted WUE_i / Actual WUE_i (μmol CO_2 mol^{-1} H_2O)
Severe stress	Callet	0.075 ± 0.033	119.8 ± 7.7	100.8	19.0
	Gorgollasa	0.062 ± 0.029	106.4 ± 8.1	103.2	3.2
	Giro ross	0.046 ± 0.009	107.6 ± 10.6	105.940	1.7
	Callet Blanc	0.059 ± 0.021	101.6 ± 23.8	103.7	-2.1
	Moll	0.065 ± 0.041	100.1 ± 32.1	102.6	-2.5
	Vinater Blanc	0.075 ± 0.022	83.5 ± 12.7	100.8	-17.4
Moderate stress	Espero	0.103 ± 0.028	117.6 ± 15.5	95.3	22.3
	Galmeter	0.108 ± 0.034	111.1 ± 15.7	94.2	16.9
	Valent Blanc	0.145 ± 0.049	100.4 ± 12.1	86.0	14.4
	Valent Negre	0.089 ± 0.033	106.9 ± 21.6	98.0	8.9
	Tempranillo	0.138 ± 0.080	95.8 ± 11.8	87.8	8.1
	Manto Negro	0.120 ± 0.015	90.6 ± 6.6	91.6	-1.1
	Merlot	0.133 ± 0.058	86.4 ± 15.6	88.9	-2.5
	Cabernet	0.141 ± 0.067	82.0 ± 15.3	86.9	-4.9
	Sabater	0.119 ± 0.038	86.4 ± 7.9	91.8	-5.5
	Argamusa	0.079 ± 0.022	81.1 ± 17.6	100.1	-18.9
	Syraz	0.116 ± 0.048	68.7 ± 6.0	92.5	-23.8
No stress	Escursac	0.183 ± 0.083	80.6 ± 6.4	76.7	3.9
	Vinater Negre	0.150 ± 0.073	86.9 ± 12.8	84.8	2.1
	Garnacha	0.204 ± 0.067	72.8 ± 15.1	70.9	2.0
	Chardonnay	0.227 ± 0.100	64.0 ± 10.7	64.5	-0.5
	Macabeo	0.154 ± 0.064	75.2 ± 14.0	83.9	-8.7
	Malvasia	0.153 ± 0.063	71.3 ± 15.6	84.2	-12.9

Table 4. Values of g_s, actual WUE_i, predicted WUE_i (calculated from figure 4B) and ratio between actual and predicted WUE_i of those tempranillo clones out of confidence interval (95%). Data are mean ± SD (n=6). Data are mean ± SD (n=3-6)

Group	Tempranillo Clones	g_s (mol m^{-2} s^{-1})	Actual WUE_i (µmol CO_2 mol^{-1} H_2O)	Predicted WUE_i (µmol CO_2 mol^{-1} H_2O)	Predicted WUE_i/ Actual WUE_i (µmol CO_2 mol^{-1} H_2O)
Severe stress	807	0.054 ± 0.008	147.3 ± 11.6	140.5	4.8%
	RJ78	0.063 ± 0.018	126.5 ± 14.5	134.8	-6.2%
	RJ43	0.052 ± 0.025	131.4 ± 2.7	141.8	-7.3%
Moderate stress	814	0.096 ± 0.020	136.3 ± 10.6	116.2	17.3%
	260	0.100 ± 0.017	131.0 ± 2.6	114.4	14.5%
	280	0.116 ± 0.022	121.5 ± 15.2	106.2	14.4%
	560	0.107 ± 0.047	124.7 ± 34.0	110.5	12.9%
	518	0.091 ± 0.023	132.4 ± 9.3	119.0	11.3%
	RJ26	0.086 ± 0.031	132.9 ± 22.8	121.8	9.0%
	RJ78	0.144 ± 0.053	100.6 ± 14.9	94.0	7.1%
	843	0.083 ± 0.025	129.0 ± 5.5	123.5	4.4%
	VN32	0.125 ± 0.016	97.0 ± 6.5	102.3	-5.2%
	RJ78	0.108 ± 0.045	99.0 ± 17.4	110.0	-10.1%
	VN1	0.149 ± 0.000	79.8 ± 3.0	91.7	-13.0%
	1041	0.083 ± 0.022	107.6 ± 4.1	123.3	-12.8%
	RJ51	0.093 ± 0.029	99.3 ± 18.5	117.9	-15.9%
No stress	RJ26	0.199 ± 0.023	88.8 ± 2.3	75.2	18.1%
	1089	0.162 ± 0.051	96.6 ± 25.0	86.9	11.2%
	1084	0.196 ± 0.070	81.7 ± 18.8	76.1	7.5%
	VP28	0.267 ± 0.080	66.7 ± 10.2	62.9	6.0%
	VN69	0.198 ± 0.064	68.7 ± 17.0	75.6	-9.3%
	VN1	0.178 ± 0.060	74.3 ± 9.5	81.5	-9.0%
	VN33	0.210 ± 0.051	64.9 ± 12.7	72.5	-10.5%
	VN31	0.164 ± 0.035	77.5 ± 8.5	86.2	-10.1%
	RJ43	0.185 ± 0.035	70.0 ± 6.8	79.3	-11.7%
	VN3	0.161 ± 0.044	75.3 ± 21.2	87.5	-13.9%

Table 5. Mean value, standard deviation (s) and coefficient of variance (CV) of g_s and WUE_i measured in grapevine cultivars and Tempranillo clones.

	g_s (mol^{-1} H$_2$0 m^{-2} s^{-1})			**WUE_i** (µmol CO$_2$ mol^{-1} H$_2$O)		
	Mean	**s**	**CV**	**Mean**	**s**	**CV**
Grapevine cultivars	0.159	0.1	60%	91.75	29.41	32%
Tempranillo clones	0.114	0.06	51%	108.6	28.3	26%