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The intra-cultivar variability on water use efficiency at different water status as a target selection in grapevine: Influence of ambient and genotype

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ABSTRACT

To face the challenges induces by the climate change a better water use in agriculture is needed. One of the ways to get it is the genetic selection and breeding programs of genotypes focused on their water use efficiency (WUE). Grapevine crop is commonly growing under water stress conditions; to improve their WUE is a general goal for viticulture. In this study, we show the variability in WUE among clones of Tempranillo, cvar, grown under both pot and field conditions, all submitted to a large range of water availability, and along three consecutive years. Leaf net photosynthesis rate (A_n), stomatal conductance (g_s) were measured, and intrinsic water use efficiency (WUE_i) was computed as the ratio A_n/g_s. Firstly, we observed that the WUE_i showed important variations among clones. Field-growing plants consistently showed higher WUE_i than pot growing ones, and an important year effect was observed. The differences among genotypes were significant in pot conditions, but not in field. Nevertheless, the present results show intra-cultivar variability in Tempranillo in WUE_i, and therefore the possibility to build a selection program based in this criterion.

1. Introduction

Regarding the IPCC predictions for an increase of the average temperatures and the frequency of extreme drought and/or warm events (IPCC, 2014), the improvement of the crops water use efficiency (WUE) has become a priority in basis and applied research. In the case of the viticulture, this topic is of special interest due to the wide distribution of this crop in semi-arid regions (Flexas et al., 2010; Zarrouk et al., 2016). To achieve an improvement of vineyard WUE there are two main ways: the agronomic techniques and the genetic improvement (Medrano et al., 2015). The agronomic techniques include the irrigation management and scheduling (Cifre et al., 2005), alternative soil management techniques as cover crops and mulching (Nguyen et al., 2013; Pou et al., 2011) and different pruning techniques (Serra et al., 2014), among others

The genetic improvement is based on the large diversity of the genus *Vitis* (Carbonell-Bejerano et al., 2013), which allows its cultivation in humid and dry climates (Medrano et al., 2018; This et al., 2006). Some cultivars are usually considered more drought tolerant than others, and a wide variability in WUE is already reported (Bota et al., 2016). The WUE reflects the balance between carbon gain and the associated cost in water, and can be measured at different spatial and temporal scales (Medrano et al., 2018). Previous studies tried to quantify this variability measuring WUE_i in a certain leaf as representative of the plant (Martorell et al., 2015; Tomás et al., 2014) or estimating WUE_i as the surrogate character isotope 13 C discrimination in biomass ($\delta^{13}C$) (Bchir et al., 2016; Santesteban et al., 2015) in different grapevine cultivars under field conditions.

However, the particularities of the wine market, dominated by Protected Designations of Origin (DOP) and Protected Geographical Indications (IGP) in Spain (equivalent to VQPRD in France), prevent the replacement of some authorised varieties by others. For this reason, different clonal selection programmes have been done since last century with success as much in the private as in the public sector. Some of these achievements were addressed to improve productivity, higher diseases resistance or particular adaptation to limiting environmental characteristics (Bois et al., 2016).

In this context, Tempranillo cultivars shows a huge distribution in Spain and other countries, with more than 200.000 Ha cultivated and it is in expansion (Ibáñez et al., 2015). This cultivar is allowed in 28 DOP

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(Protected Denomination of Origin) and, at present, there are 49 certified clones of Tempranillo cv (Ibáñez et al., 2015). Moreover, in the last decade variability in Tempranillo clones has been shown by Rubio and Yuste (2004) who founded differences between Tempranillo clones in ampelographic description, by Revilla et al. (2009) for anthocyanin fingerprint, Arrizabalaga et al. (2018) in the response of berry sugar and anthocyanin accumulation to elevated temperature, among others (Tello et al., 2018; Torres et al., 2018). In the case of the WUE_i, our group demonstrated in a Tempranillo clonal collection that in respect to a wide cultivar collection, the variability is at least an 80% of the showed by cultivars (Tortosa et al., 2016).

In this study, we evaluate the WUE_i response of seven Tempranillo clones measured at different water status and experimental conditions in order to estimate the effect of environmental conditions on the WUE_i and to evaluate this effect on the comparison of the cultivar performance.

2. Material & methods

2.1. Plant material and water status

Seven Tempranillo clones, three commercial clones (RJ43, RJ51 and RJ78) and four experimental genotypes (232, 1048, 1052 and 1084), were studied during three consecutive experimental campaigns at field conditions (2015-2017). In addition, during the 2017 a pot experiment was made. Field campaigns were done in the experimental field of the ICVV (Instituto de las Ciencias de la Vid y el Vino, Logroño, La Rioja, Spain) and in the experimental field of Viveros Provedo, a commercial nursery (Viveros Provedo S.A., Logroño, La Rioja, Spain). We measured plant water status and gas exchange parameters in 5-6 plants per clone, once per campaign except year 2016 (measured tree times in June, July and August respectively). Measurements were done in two sites: at the ICVV public clonal collection field (experimental clones), and in Viveros Provedo (commercial clones). All clones used in the different experiments were grafted onto 110-Richter rootstock, trained as a double cordon, similarly pruned, and managed on a standard procedure.

The pot experiment was carried out at the experimental field of University of Balearic Island (UIB), with the plants grafted onto same rootstock (110-R). Plants were in 20 L pots (5 plants per genotype), filled with organic substrate and perlite mixture (5:1). Plants were irrigated three times per week from May, until plant shoots were about 1.5 m high. Two weeks later the irrigation dosage was progressively reduced for one month to get a wide range of soil water stress.

2.2. Gas exchange measurements

Leaf net photosynthesis (A_n) and stomatal conductance (g_s) were measured in a fully exposed mature leaf (one per plant, n = 4-6 per clone). All determinations were done between 10:00 and 13:00 h (local time) using an infrared open gas analyser system (Li-6400xt, Li-cor, Inc., Licoln, Nebraska, USA). The CO_2 concentration inside the chamber was 400 µmol CO_2 mol^{-1} air, PAR was always above saturation levels. WUE_i was calculated as the ratio between A_n and g_s. For pot experiment, measurements were performed every week at different plant water status until the stomatal conductance decreased to 0.05 mol H_2O m^{-2} s^{-1}. Then irrigation was applied.

2.3. Data treatment of the WUE_i – g_s relationship

The strong and negative WUE_i - g_s relationship is well known and prevents to compare genotypes under different water status (Medrano et al., 2018). The results obtained were arranged in three categories according to previous reports (Jara-Rojas et al., 2015; Medrano et al., 2002): Plants under non water stress conditions (g_s > 0.15 mol H_2O m^{-2} s^{-1}), moderate water stress (g_s between 0.15 – 0.075 mol H_2O m^{-2} s^{-1}) and severe water stress (g_s < 0.075 mol H_2O m^{-2} s^{-1}).

2.4. Statistical analysis

All statistical analyses were performed using R (Team, 2014). Growing conditions (pot *versus* field) and genotypes were compared based on differences in their WUE_i – g_s regressions slopes using ANCOVA from the 'car' package (Fox and Weisberg, 2011). In some cases, to increase the robustness of the comparisons, we transformed the data with natural logarithm in order to increase the linearity of each regression slope. Differences in slopes were accepted with p-value < 0.05. Comparison one to one were performed with "cld" analysis from the 'emmeans' package (Lenth, 2018).

3. Results

3.1. Plant water status and WUE

Considering all the genotypes measured in all experimental conditions, g_s values ranged between 0.05 and 0.45 mol H_2O m^{-2} s^{-1}, showing a large difference in plant water status. The values of net CO_2 assimilations (A_n) ranged from 3 to 21 µmol CO_2 m^{-2} s^{-1}, which resulted in a large variability of WUE_i, ranging from 20 to 160 µmol CO_2 mol^{-1} H_2O. The WUE_i was strongly and negatively related to g_s, as shown in Fig. 1A (R^2 = 0.75). The mean values of WUE_i in each group (non- stressed, moderate and severe water stress, see M&M section) were 60, 90 and 115 µmol CO_2 mol^{-1} H_20, respectively. The regression between WUE_i – g_s for each water status (Fig. 1B), showed divergences in the magnitude of the effect and the level of significance. In stressed plants, the slope of WUE_i – g_s was higher and the p-value lower, conversely to observed in

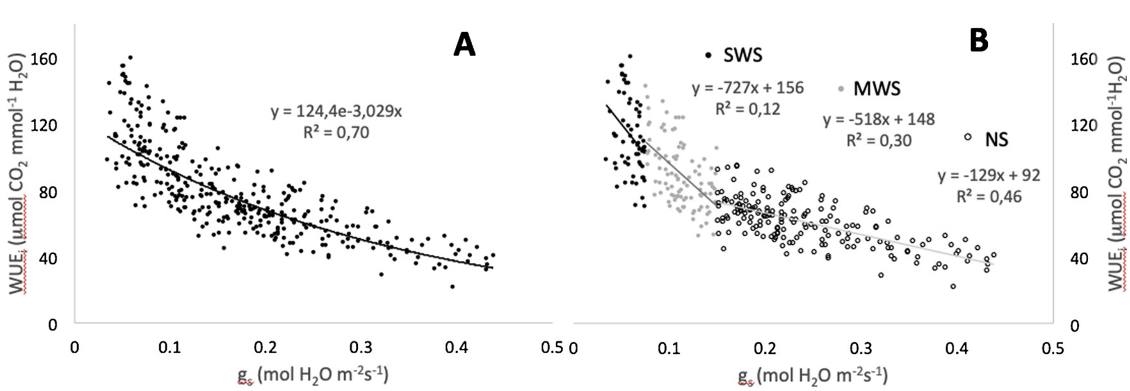

Fig. 1. General correspondence between stomatal conductance (g_s) and intrinsic water use efficiency (WUE) (A) and individual relationship between both variables for each water status interval (B). Data are all the replicates, grown at field and in pot conditions and at different water status in the three years of experiment (NS: Non-stressed; MWS: Moderate water stress; SWS: Severe water stress).

Table 1
The slope between the natural logarithm of the WUE_i against g_s and the estimated WUE_i estimated by the model for each g_s range. Letters means significant differences at $p < 0.05$ following Post-Hoc test.

Year	Slope $WUE_i - g_s$	g_s range ($mmol\ m^{-2}\ s^{-1}$)		
		0.075	0.150	0.350
2015	−4.11 + -0.36a	120.5a	88.6a	38.9a
2016	−2.88 + -0.18b	107b	86.5a	48.6a
2017	−2.31 + -0.48b	90.6c	75.9b	47.8a

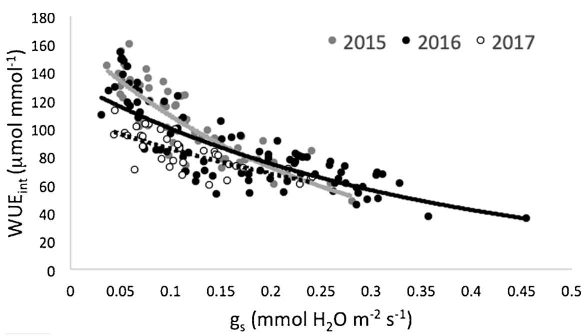

Fig. 2. WUE_i-g_s relationship under field conditions only, for the three years of measurements, with 2015 in grey, 2016 in black and 2017 in open circles and dotted regression line.

non-stressed plants.

3.2. The year effect on WUE at field conditions

Under field conditions, all the genotypes were measured each year and this allowed to test the year effect in the $WUE_i - g_s$ relationship. We observed that the slope of this relationship in 2015 was significantly different from those of 2016 and 2017 (p-value < 0.05, Table 1). Generally, plants of 2015 showed a higher WUE_i, and especially at low g_s, but at g_s larger than 0.12, WUE_i was similar between all years (Fig. 2).

3.3. Pot vs. field conditions

To compare the effect of the growing conditions (field *vs* pot), we evaluated the WUE response to g_s in each situation along a huge range of g_s. Under field conditions the minimal g_s were around 0.045 mol H_2O m^{-2} s^{-1} and the maximum g_s was 0.3, while in pots the maximum reached 0.45 mol H_2O m^{-2} s^{-1} (Fig. 3A). The $WUE_i - g_s$ relationship showed differences between pots and field conditions. To confirm this

Table 2
Comparison between pot and field WUE_i values calculated by the natural logarithm regression at g_s values representative of non-stressed, moderate, and severe stressed conditions.

g_s (mmol $H_20\ m^{-2}s^{-1}$)	Field WUE_i (µmol CO_2 $mmol^{-1}H_2O$)	Pot WUE_i (µmol CO_2 $mmol^{-1}H_2O$)	Difference
0.075	107.5 ± 1.02	86.9 ± 1.02	19% ***
0.15	85.8 ± 1.01	71.3 ± 1.01	17% ***
0.3	54.6 ± 1.03	48.1 ± 1.02	12% ***

[a] indicates significant differences between points (p-value < 0.001).

observation, we linearized the regressions using the natural logarithm (Fig. 3 B). Analysis of co-variance shows a strong effect on the intercept of the two regressions (p-value < 0.0001) and a significant difference between the two slopes (p-value < 0.05). At low g_s, difference between the WUE_i measured in field and pot conditions was higher, and this difference was reduced with an increase of g_s. With a g_s of 0.1 mol H_2O m^{-2} s^{-1}, the mean value of WUE_i in pot conditions was 20% lower than the field conditions, and at g_s of 0.3 mol H_2O m^{-2} s^{-1} this difference was around 10% (Table 2). Thus, for similar conditions of water stress (estimated with g_s), the WUE_i was clearly higher for field growing plants. We tested this differences removing the year 2015, that showed particularly high WUE_i, and we found the same differences in intercept (higher WUE_i under field conditions), but not in slope.

3.4. Genotype variability on WUE

To compare the genotypes individual response, a linear logarithm regression of each genotype for the different g_s was done. Comparing the performance of each individual genotype under field conditions only, (Table 3), the $WUE_i - g_s$ showed R^2 between 0.25 and 0.73 (average = 47.7). Regarding the lower R^2 and the higher standard errors in the slope estimations, no differences were found in the slopes between genotypes under field conditions.

Under pot conditions, the R^2 varied between 0.48 and 0.85 (average = 75.1). The management of the irrigation system allowed to measure a wide range of g_s with a slightly higher amplitude than under field conditions, with maximum values reaching 0.45 mol H_2O m^{-2} s^{-1}. The resultant slopes varied with the same amplitude than in field conditions, ranging between -3.7 to -2.2 (Table 3). In this case, the g_s x genotypes interaction factor of the ANOVA was significant (p-value < 0.01). This interaction was due to a significant difference in slope between genotypes 1052 and RJ78.

Comparing the regression slopes between field and pot conditions inside each genotype, there was no difference in slopes between field and pot conditions. Thus, we repeated the comparison between genotypes but grouping field and pot data of each of them, and the

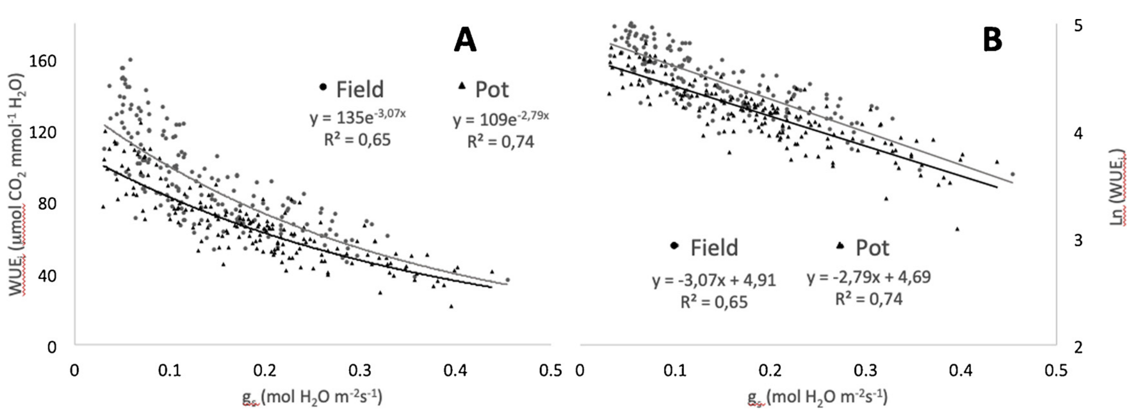

Fig. 3. Comparison between field (circles) and pot (triangles) conditions of WUE_i-g_s relationship (A) and linearized regressions using the natural logarithm of WUE_i (B). Data are individual measurements of each clone measured during the three years of experiments.

Table 3
Pearson coefficient (R^2 and slopes) of the gs-WUE_i regression of each genotype in field and pot conditions. Different letters indicate significant differences (p-value < 0.05) among genotypes in each comparison..

	Field conditions		Pot conditions		Field + pot	
Genotype	R^2	Slope	R^2	Slope	R^2	Slope
232	0.25^{*}	-4.28 ± 1.62	0.72^{***}	$-2.40 \pm 0.27^{a,b}$	0.71^{***}	$-3.40 \pm 0.30^{a,b}$
1048	0.58^{***}	-2.62 ± 0.47	0.76^{***}	$-3.10 \pm 0.33^{a,b}$	0.65^{***}	$-3.16 \pm 0.32^{a,b}$
1052	0.52^{***}	-3.13 ± 0.75	0.88^{***}	-3.68 ± 0.34^{a}	0.79^{***}	-4.03 ± 0.35^{a}
1084	0.73^{***}	-2.95 ± 0.4	0.78^{***}	$-3.00 \pm 0.41^{a,b}$	0.67^{***}	$-2.96 \pm 0.34^{a,b}$
RJ43	0.6^{***}	-2.68 ± 0.37	0.79^{***}	$-2.98 \pm 0.35^{a,b}$	0.71^{***}	$-3.16 \pm 0.27^{a,b}$
RJ51	0.62^{***}	-2.73 ± 0.44	0.48^{***}	$-2.18 \pm 0.57^{a,b}$	0.55^{***}	$-2.50 \pm 0.35^{a,b}$
RJ78	0.58^{***}	-3.02 ± 0.49	0.85^{***}	-2.26 ± 0.16^{b}	0.79^{***}	-2.71 ± 0.18^{b}

** P < 0.01.
[b]P < 0.05.
[b]P < 0.001.

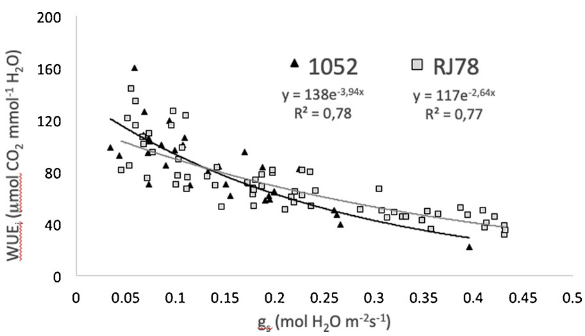

Fig. 4. Comparison of genotypes RJ78 (grey squares) and 1052 (black triangles) in WUE_i slopes considering the whole range of stomatal conductance combining field and pot data.

Table 4
Comparison of genotypes 1052 and RJ78 in WUE_i calculated by the natural logarithm regression at different g_s values.

g_s (mmol $H_20\ m^{-2}s^{-1}$)	1052 µmol CO_2 mmol$^{-1}H_2O$)	RJ78 µmol CO_2 mmol$^{-1}H_2O$)	Difference	
0,05	115.3 ± 1.04	104.3 ± 1.04	10%	.
0,1	94.3 ± 1.03	91.1 ± 1.03	3%	
0,2	63.1 ± 1.04	69.4 ± 1.02	10%	**
0,3	42.2 ± 1.06	52.9 ± 1.03	26%	***

P < 0.1, * P < 0.05, ** P < 0.01, *** P < 0.001.

interaction factor of the ANCOVA was significant (p-value < 0.05). Significant differences were found again between genotypes 1052 and RJ78, with slopes between -4 and -2.50 (Table 3). Then, genotype 1052 was clearly more conservative in the use of water under non-stressed conditions. It is important to note that the difference in slope was not accompanied by a systematic higher WUE of the genotype 1052 compared to the RJ78 (Fig. 4). At low water availability, the 1052 showed higher WUE_i than the RJ58, while at higher g_s the opposite was observed (Fig. 4 and Table 4).

4. Discussion

The performance of seven genotypes of Tempranillo cultivar were compared in two different sites, for three years, and one year in pot conditions along a wide range of soil water availability conditions. We found that, globally, plants grown in field conditions showed higher WUE_i compared to pot conditions, and these differences increased when water availability become more limiting. Interestingly, two of those genotypes showed a statistically different WUE-g_s slopes under pot conditions, demonstrating that a genetic diversity in WUE_i exist at the clonal level (inside the same cultivar).

The A_n, g_s and WUE_i resultant from this study were comprised inside the range of accepted values for the grapevine (Bota et al., 2016; Martorell et al., 2015; Medrano et al., 2002). The water management allowed us to obtain a wide range of g_s, that confirm that plants showed different water status, from no stress to severe water stress, in both field and pot conditions as was observed by Medrano et al. (2002). Data of stem water potential measured at midday confirmed also this observation (not shown). Overall, the wide range of g_s allowed us to relate WUE_i – g_s through regressions lines with a good estimate of the slope. This enabled to compare the slopes between themselves to highlight environmental and genetic differences.

4.1. Year effect

The capacity to maintain the WUE_i at low g_s was clearly higher during the year 2015 in respect to 2016 and 2017, showing an interesting "year effect". This could be related to a highest water stress conditions during this year compared to 2016-2017. Analysing climatic conditions during grapevine growth, (Table 5), precipitations were of 2.6 mm in May 2015, while there were of 19.9 mm and 74.6 mm in 2016 and 2017 respectively. Month of May corresponds to the leaf formation, therefore could influence the morphology or biochemistry of the measured leaves. There is poor information about the impact of the climate conditions during the leaf development and their consequences in WUE_i. We can nevertheless hypothesise that the strong water stress conditions in May 2015 have influenced the leaf formation (narrow vessels, higher LMA or others factors) that could influence leaf performances at low g_s ones mature.

4.2. Field vs. pot responses

Respect to the growing conditions, field *versus* pot, data showed an important effect on the WUE_i - g_s slope, with a systematic higher WUE in the field than in pot for a given g_s. These differences increased as

Table 5
Climatic conditions in Logroño during the three experimental years (https://www.larioja.org/agricultura/es/informacion-agroclimatica/red-estaciones-agroclimaticas-siar)..

	Year	Tmed (ºC)	P (mm)	Eto (mm)
May	**2015**	16.3 ± 3.4	2.6	142.5
	2016	14.8 ± 2.5	19.9	130.1
	2017	17.4 ± 3.7	74.6	145.3
June	**2015**	20.3 ± 3.8	42.8	170.7
	2016	19.3 ± 3.4	12.5	165.3
	2017	21.3 ± 4	42.4	167.3
July	**2015**	23.2 ± 2.9	34.9	197.8
	2016	21.5 ± 2.8	34.4	174.8
	2017	22.1 ± 3.1	15.7	190.3

water stress become more severe (at lower g_s). The highest WUE_i at a given g_s in field can be due to a slightly higher leaf photosynthetic capacity of field plants than the plants grown in pots (Poorter et al., 2012). This could be related with leaf biochemistry, higher mesophyll conductance, or increased leaf hydraulic conductance. It is also highly probable that this change could be related to the deeper root system of the field plants (Bota et al., 2001). On the other hand, it is poorly probable that the difference comes from a lack of nutrient of the potted plants since pots were irrigated with Hoagland solution every two weeks. It is also noticeable that plants grown in pots were subjected to well-watered conditions until June, and then watering was decreased at the desired intensity to obtain the targeted g_s. On the other hand, plants under field conditions were grown under a progressive drought along the summer, while in potted plants water stress occurs quickly (Escalona et al., 1999). This means that the measured pot leaves during the summer could have a lower LMA because the leaves expanded under well-watered conditions. A higher LMA induce thicker and/or denser leaves, and then with more photosynthetic tissue per area unit (Poorter et al., 2012).

Finally, a question remains: does the genetic variability revealed in pots really exists under field conditions?. If it is the case, increased number of replicates under field conditions will make such differences more evident, but for to apply physiological selection criteria the sample number results critical.

4.3. Genotype variability

Under field conditions, no statistical differences between genotypes were found. This was due to higher standards errors in the slope estimations because of lower R^2 of the WUE_i – g_s. First, for some of the genotypes, the range of g_s was lower in field than in pots. This reduce the robustness of the slope estimations in many cases.

On the other hand, in pot conditions, the lower data dispersion (higher R^2) allowed to detect the clonal variability, where genotypes 1052 and RJ78 were identified as statistically different in their WUE_i – g_s regression slopes. This fine adjustment of the WUE_i – g_s regressions corresponds with the fine control of the water management system and the uniformity of soil conditions among plants. As was mentioned, the highest range of g_s also helped to reinforce the regression slope estimations, allowing the ANCOVA to find significant differences. This ensure that the differences between clones of Tempranillo were real and robust. The absence of differences in regression slope within each genotype between field and pots, allowed us to group field and pot data for each genotype, increasing the robustness of each slope estimation. The resultant regression obtained within each genotype shown again that genotype 1052 have significant lower slope than RJ78, reinforcing the idea that an intra-cultivar variability exists. This difference is slope could be associated to a higher photosynthetic capacity of the genotype RJ78. Many factors can explain this difference, like higher nitrogen content, higher LMA, or higher mesophyll conductance (Tomás et al., 2014).

It is important to note that the difference in slope was not accompanied by a systematic higher WUE of the genotype 1052 compared to the RJ78 (Fig. 4). Under low water availability, the genotype 1052 shows higher g_s than the genotype RJ58, while at higher g_s, it is at the reverse (Table 4). This means that each genotype could perform better WUE than the other one, depending of the water availability conditions. Nevertheless, the physiological underlying mechanism responsible of those differences remains unknown. Those differences are on the basis of measurements at the leaf scale. It is necessary to clarify in which extent those observed differences are also reflected at the whole plant scale, and how they are related with others agronomic parameters like harvest production and grape quality.

5. Conclusion

We confirmed that it is possible to find a genetic variability of WUE_i between clones of the Tempranillo cultivar, even though an important effect of environment and growing conditions is present. We also highlighted the fact that pot and field conditions do not lead to the same values of water use efficiency, and that specific climatic conditions during leaf growth influence this behaviour. When this environmental variability was reduced, in pots experiments, a significant genetic variability was detected enabling the identification of certain genotypes with higher and lower WUE. The joint analysis of pot and field data showed clear coincidences among the two set of data for contrasting WUE values of the analysed genotypes. Future studies could enlarge the panel of genotypes characterised, and focus on the underlying processes explaining the observed differences in water use efficiency.

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