LOOKING FOR MORE WATER USE EFFICIENT GENOTYPES. A KEY FOR A SUSTAINABLE VITICULTURE

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Abstract

Aim: Grapevine has traditionally been widely cultivated in drylands. However, in recent decades, a significant part of the viticulture all over the word and specifically in Mediterranean basin, is being irrigated. In recent years, due to climate change, among other reasons, the available natural water resources have been reduced substantially compromising the sustainability of viticulture, especially in the most arid areas. Therefore, it is necessary to search for genotypes with greater water use efficiency (WUE not only among varieties but also, between clones of the same variety).

Methods and Results: In this work, 23 clones of cv. Tempranillo were evaluated during five consecutive years in two experiments. First, a three-year field experiment determining the variability in WUE by measuring gas exchange parameters. Second, a two-year experiment in pots, analyzing the response of those Tempranillo clones to different degrees of soil water availability. Different growth parameters, leaf gas exchange rates, and biomass production were measured. Field data of leaf exchange rates and derived parameters showed a wide variability among clones in WUE up to 80% to that previously achieved comparing different cultivars. These differences appear to be due to differences in photosynthesis capacity rather than to a more efficient control of water loss. Pot experiments reveal differences among clones in biomass production and gas exchange parameters as indicators of plant water use efficiency. A joint analysis of pot and field data showed a consistency in higher and lower WUE genotypes, although significant environmental condition effects were present.

Conclusions: The whole analysis of WUE indicators quantified the degree of variability in WUE among clones, and identified the best and worst water use efficient clones in both well-watered and water deficit conditions.

Significance and Impact of the Study: These findings open new ways for future research focused on the physiological basis of the variations in WUE, and can also be extended to other reputed drought-tolerant cultivars.

Keywords: *Vitis vinifera*, clones, Tempranillo, drought, water use efficiency

Introduction

Climate change (CC) is one of the most important challenges for the future of viticulture in the Mediterranean area (van Leeuwen *et al.,* 2016). To face the consequences of climate change, different options are already in use such as moving to a higher altitude or increasing irrigation dependency (Fraga *et al.,* 2013). Another way to deal with adverse weather conditions is the replacement of plant material to choose one more adapted to the new environmental conditions (Vivin *et al.,* 2017). This could also result in a more sustainable vineyard in terms of water management. Vine genetic variability is an invaluable resource that offers a selection of cultivars with a potential higher drought or heat tolerance (Medrano *et al.,* 2018, Laucon *et al.,* 2018).

In Spain, Tempranillo is the most grown red cultivar in any wine region in the country. The wide geographical distribution of Tempranillo cv confers its high intra-cultivar variability, which is represented by up to 50 commercial clonal lines available for farmers. The variability inside one cultivar is a promising way to maintain wine sustainability in the near future (Ibáñez *et al.,* 2015). Therefore, we have explored the inherent variability of the Tempranillo cultivars in order to select clones with improved water use efficiency (WUE).

WUE is commonly accepted as a reliable criteria to evaluate the water dependency of one genotype, cultivar or clone, referred mainly to water productivity (production per unit of water used). Such WUE can be measured at different evaluation scales like the plant production in terms of biomass or crop production per unit of water applied (whole plant WUE; WUE_{wp}); or at the leaf scale, evaluating the net carbon gain (A_N) per water transpired or more specifically per unit of stomatal conductance (g_s) (intrinsic WUE; WUE_i), as well as surrogate measures such as $\delta^{13}C$ discrimination (Flexas *et al.,* 2010; Bchir *et al.,* 2010; Santesteban *et al.,* 2012). However, the relationships between the estimated WUE at different scales shows variable agreement (Tomás *et al.,* 2014). Despite these apparent differences between measuring scales, the genetic variability in terms of WUE has been widely studied within grapevine cultivars (Bota *et al.,* 2001; Tomás *et al.,* 2012; Costa *et al.,* 2012). So, comparative measurements of leaf gas exchange parameters seems a promising way to characterize genotype WUE both under pot and field conditions therefore allowing for the ranking of plant material by WUE (Morales *et al.,* 2020).

Clonal variability was early used by commercial nurseries to obtain new certified material resistant to fungal or bacterial infection, or differentiated by production or quality (Rühl *et al.,* 2004). Nowadays, there is an increasing interest to show the performance of some clones under simulated climatic change conditions as well as to establish large clone collections to conserve the intra-cultivar variability and to use molecular markers to identify clonal differences (Grimplet *et al.,* 2019). Because the wine industry regulations make variety replacement difficult, we consider the evaluation of genetic variability for WUE among different clones of Tempranillo, the most important and widespread Spanish wine making variety. In the present work, we study the behavior up to 40 Tempranillo clones and biotypes subjected to different soil water availability. The experiment was carried out in two field collections and in pot conditions with water availability control. During three consecutive years different parameters related to water status, plant growth, and gas exchange regulation were measured in order to establish a provisional ranking of clones based on WUE at leaf scale and eventually to explore the capacity to select the ones with higher and lower WUE.

Materials and Methods

The field experiments were conducted in two experiment sites, both in Northern Spain. The first one, in the experimental field of the ICVV (Instituto de las Ciencias de la Vid y el Vino, Logroño, La Rioja, Spain), called "La Grajera". In this site, eleven clones (232, 243, 360, 1048, 1052, 1078, 1084, 1371, RJ43, RJ51, RJ78) were measured during five consecutive years. The second site was located at the Roda estate (Bodegas Roda, Haro, La Rioja, Spain), where twelve clones (6, 108, 137, 156, 166, 178, 203, 215, 326, 336, 365, 452) were measured during three consecutive years. At both sites, plants were grafted onto 110-Richter rootstock, trained as a double cordon system in La Grajera, and head-trained bush system in Haro. The vine density in La Grajera were 2600 plants Ha^{-1} and in Haro 3300 plants Ha^{-1}.

The pot experiment was carried out at the experimental field of University of Balearic Island (UIB), with the same clones grafted onto the 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 1.5m high. Two weeks later irrigation was progressively reduced for one month to get a wide range of soil water stress.

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 gs. For the 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}. The results obtained were arranged in three categories according to previous reports (Medrano *et al.,* 2002): Plants under non water stress conditions (gs>0.15 mol H_2O m^{-2} s^{-1}), moderate water stress (gs between 0.15 – 0.075 mol H_2O m^{-2} s^{-1}) and severe water stress (gs<0.075 mol H_2O m^{-2} s^{-1}). The characterization of the WUE_i among clones was done following the method described by Tortosa *et al.* (2016). A general relationship between WUE_i and gs was obtained, as expected. Provided such a relationship shows a high regression coefficient, the WUE_i observed value for a determined clone was compared to the expected for the same gs value obtaining the residual value for each genotype as percentage (residual clone / predicted clone).

All statistical analyses were performed using R. 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 using a natural logarithm in order to increase the linearity of each regression slope. Differences in slopes were accepted with p-value< 0.05.

Results and Discussion

A_N/g_s is a widely accepted parameter to characterize the WUE in a leaf, but it's largely influenced by soil water availability. We measured leaf net photosynthesis (A_N) and stomatal conductance (g_s) in different experimental fields during different phenological stages to measure the performance of each genotype in a wide range of water status (Tortosa, 2019a; 2019b). Plotting the WUE_i values against their corresponding g_s (Figure 1A), a general response curve was obtained and the particular position of the WUE_i value of each genotype was analysed according to the residual analysis of each genotype average in respect to the general regression curve following Tortosa *et al.* (2016). The WUE_i variation, ranged from 70 to 145 µmol CO_2 mol H_2O^{-1} (Figure 1A). Following this method to characterize the genotype behavior, the general soil moisture effect (estimated as g_s) was avoided and the WUE_i variability was reduced to a 30% (Figure 1B) (Medrano et al., 2018). The results of this field evaluation and differences among genotypes were deeply discussed in Tortosa *et al.* (2019b).

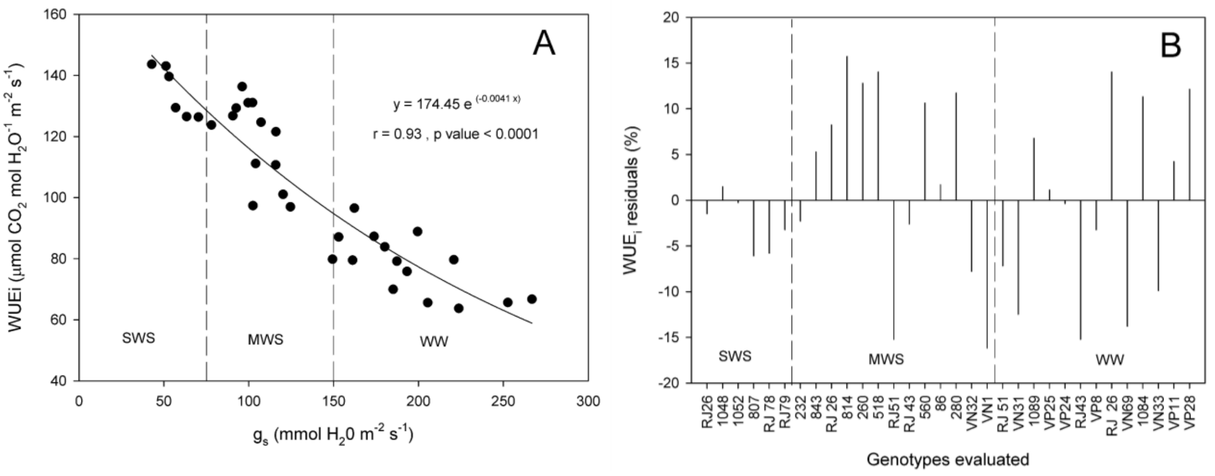
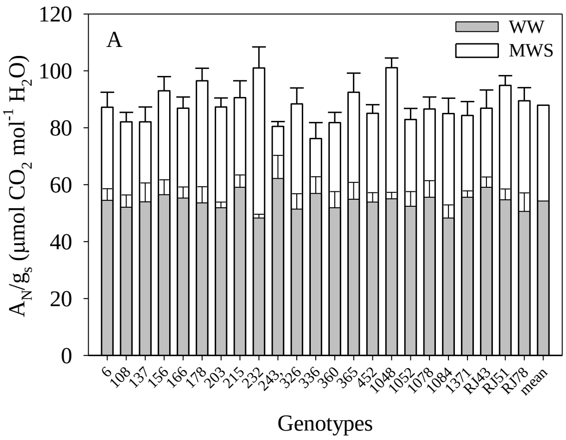


Figure 1: Relation between stomatal conductance (g_s) and intrinsic water use efficiency for calculation of residual values of WUE used for clone ranking procedure (A) and the difference between predicted and actual values of WUE_i in percentage for each clone under well-watered conditions (WW) and moderate (MWS) and severe (SWS) water stress conditions (B) From Medrano *et al.* (2018).

Concurrently, potted experiments performed using the same clones allowed more effective water status control, reducing the effect of variability and non-controlled interferences such as different individual root extension or particular vegetative development conditions. Contrary to WW, in MWS conditions we found again a high variability in WUE_i, ranging from 75 to 100 µmol CO_2 mol^{-1} H_20 in MWS (Figure 2A). Also, genotypes showed a wide range of variability in biomass production (from 125 to 75 g dry mass plant^{-1}) (Figure 2B). The potted experiments results were discussed in Tortosa *et al.* (2020), but as a conclusion, we confirmed that the variability found in field conditions were also reflected in pot conditions.



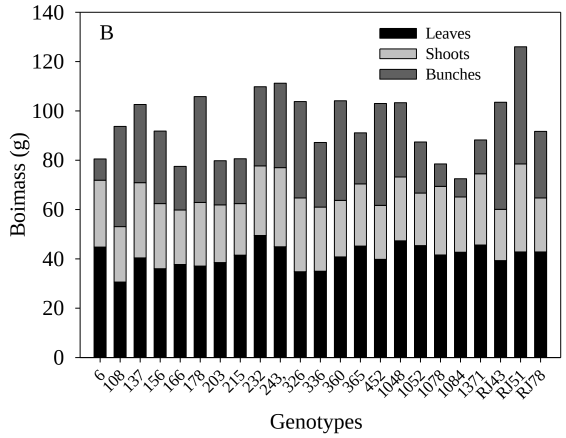


Figure 2: WUE data of each Tempranillo clon (A) under well-watered (grey bars) and moderate water stress (white bars) conditions and total dry biomass divided in leaves (black bars), shoots (grey bars) and bunches (dark bars) (B) measured at the end of experiments 2018 for each genotype (N = 5). Data are mean of five replicates. From Tortosa *et al.* (2020).

Finally, Table 1 shows a comparison among genotypes performance under field and pot conditions and for two different plant water status. Total WUE_i variability shown by this large collection of genotypes was reduced in the pot experiment, especially in WW status. This reduction is probably due to more uniform climatic and edaphic conditions compared to the field experiments. In general, no relationship between pot and field results was achieved. Despite this, it was possible to identify some similar genotypes in the two water status conditions. For example, in WW conditions, genotype 6 recorded the higher WUE_i compared to the average under field and potted conditions, contrary to genotypes 326 and 215. In MWS conditions, genotypes 108, 232 and 1052 showed a good performance for all situations. However, the genotypes that registered lower WUE_i differed depending on the growing conditions.

Conclusions

We confirmed that it is possible to find 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 largely affected the particular WUE_i. When this environmental variability was reduced, in potted 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 some similarity among the two sets of data for contrasting WUE values of the analyzed genotypes. Future studies could enlarge the number of genotypes characterized, and focus on the underlying processes explaining the observed differences in water use efficiency.

Acknowledgments

This work was performed with the financial support from the Ministerio de Economía y competitividad (MINECO, Spain) (project AGL2014-54201-C4-1-R and AGL2017-83738-C3-1-R) and a pre-doctoral fellowship BES-2015-073331). The authors would like to thank Mr. Miquel Truyols and collaborators of the UIB Experimental Field (UIB Grant 15/2015) for their support to our experiments. We also want to thank the collaboration of Instituto de las Ciencias de la Vid y el Vino (ICVV) and Bodegas Roda S.A to provide us the access to their experimental field as the collected plant material for experimental plots.

Appendix

Table 1: (Predicted WUE_i – Actual WUE_i) divided by predicted WUE_i values of field and pot measurements.

	Field plants		Potted plants	
Genotype	**WW**	**MWS**	**WW**	**MWS**
6	9.7 ± 2	3.9 ± 2.8	1.2 ± 2.5	-4.7 ± 3.6
108	-1.1 ± 2.8	6 ± 3.8	3.1 ± 3.5	4.4 ± 5.8
137	-23.1 ± 0	-	0.1 ± 3	5.3 ± 3.9
156	-5.7 ± 0	-	-4.4 ± 3.1	5.8 ± 3.4
166	-3.2 ± 0	-	-0.3 ± 2.5	0.6 ± 2.6
178	7.3 ± 0	-	-2.7 ± 2.4	2 ± 3.5
203	5 ± 0	-	0 ± 2.1	-0.5 ± 3.5
215	-6.9 ± 0	-	-5 ± 3.2	2.2 ± 2.9
232	-	4.3 ± 3.2	0.2 ± 2.3	6.2 ± 3.5
243	-5.8 ± 0	-	7.7 ± 4.8	-0.3 ± 9
326	-15.9 ± 0	-	-4.5 ± 2.8	-3.6 ± 4.7
336	0 ± 3.6	-0.8 ± 3.8	-1.8 ± 5.5	0.9 ± 2.7
360	-12.5 ± 0	-	1 ± 2.2	-5.2 ± 2.6
365	6.8 ± 0.9	-3.5 ± 1.1	-1.8 ± 3	2.7 ± 3.6
452	-0.1 ± 0	-	-3.3 ± 2.8	1.8 ± 2.4
1048	-4.5 ± 3.4	2.4 ± 3.4	1.1 ± 2.8	6 ± 2.5
1052	1.2 ± 4.6	6.4 ± 1.9	-7.7 ± 1.7	5 ± 4.1
1078	11.1 ± 2.9	1.5 ± 2.9	0.1 ± 2.9	0.6 ± 3.1
1084	-2.4 ± 3	0.7 ± 4.4	-5.1 ± 2.7	0.9 ± 3.8
1371	-7.7 ± 5.3	3.4 ± 11.8	2.6 ± 2.9	-4.3 ± 5.7
RJ43	-2 ± 1.4	4.2 ± 1.9	-4.7 ± 3.4	-0.1 ± 4.6
RJ51	-4.4 ± 3.8	-5.2 ± 5.9	1.1 ± 3.8	9.6 ± 4
RJ78	0.5 ± 3.9	-4.3 ± 5.3	3.7 ± 2.7	2.9 ± 3.6

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