



Universitat
de les Illes Balears

DOCTORAL THESIS
2023

**EVALUATION OF GENETIC VARIABILITY ON
INTRACLONAL WATER USE EFFICIENCY IN
TEMPRANILLO CV**

Ignacio Tortosa Montojo



Universitat
de les Illes Balears

DOCTORAL THESIS
2023

**Doctoral Programme in Biomedical and
Evolutionary Biotechnology**

**EVALUATION OF GENETIC VARIABILITY ON
INTRACLONAL WATER USE EFFICIENCY IN
TEMPRANILLO CV**
Ignacio Tortosa Montojo

Thesis Supervisor: Hipólito Medrano Gil

Thesis Supervisor: José M. Escalona Lorenzo

Thesis tutor: José A. Castro Ocón

Doctor by the Universitat de les Illes Balears

Agradecimientos

Que difícil acordarse hoy de tantos, no dejarme a ninguno, porque lo importante nunca fue el destino sino la travesía... y esta fue realmente increíble.

Por supuesto, primero y antetodo agradecer a Pepe e Hipólito su dirección, su constancia y confianza en que el barco llegaría a buen puerto, a pesar de las tormentas y los oleajes, ya que su experiencia y empeño hace que hoy exista y sea posible la presente tesis. Gracias también por su paciencia infinita y por no desistir nunca...

Para continuar, agradecer también a todos los coautores de los diferentes trabajos publicados; por los buenos momentos en campo, por los consejos y por las enseñanzas al pie del cañon. A destacar muy especialmente a Cyril, Guillermo, Alicia, Pedro y, por supuesto, a Esther, hermanita de tesis. Gracias por haber hecho de las campañas una experiencia inolvidable, con licors quemados, accidentes de todo tipo, y risas, muchas risas, fruto de las borracheras de sol, hambre y agotamiento. Gracias por enseñarme desde ese primer verano la importancia de asegurar la finura de los datos y a mantener la paciencia y concentración en los momentos de estrés, por muy duras que puedan ser las condiciones; habéis sido todo un ejemplo. Bueno, y a Ismael también, que no por pequeño es menos importante...

Mi gratitud también a todo el entorno de can bum y alrededores, a todos los que compartimos despacho y camino; Toni, Mateu, Miqueles, Alicia... Drones volando, bicicletas en el laboratorio y yogures al anochecer... el buen ambiente y compañerismo tiene un valor incalculable, y me siento realmente afortunado de haber pertenecido a esta generación... Y también a Hannah el-Oeoeoeoe y a Arancha, por aguantar siempre a los "peques" con una sonrisa bondadosa y ser la voz de la consciencia entre tanto alboroto.

Los alrededores de Can bum incluyen, entre muchos otros, a Antonia, Belén, Miguel, Josefina, Álvaro, Néstor... cuya amistad trasciende las paredes de la UIB, como tantos otros, pero ellos especialmente. No olvido su consuelo y confianza en los momentos más duros, que también los hubo, porque en todo viaje hace falta un hombro en el que apoyarse cuando las fuerzas no dan para más...

Por último, y no menos importante, a mi familia, muy especialmente a aquellos que me inspiraron y animaron a seguir siempre mi sendero... y sí, me refiero a papá y a mamá, para los que siempre seguiré siendo su niño por muy lejos que pueda irme; y a Antonia, otra vez, que ha sido y es un manantial de paz y serenidad.

Index

| | |
|---|-----|
| Index | 5 |
| Chapter 1. General introduction | 13 |
| Chapter 2. Objectives | 25 |
| Chapter 3: Material and methods | 29 |
| Chapter 4: Results..... | 39 |
| 4.1. Exploring the genetic variability in water use efficiency: evaluation of inter and intra cultivar genetic diversity in grapevines..... | 41 |
| 4.2. Variability in water use efficiency of grapevine Tempranillo clones and stability over years at field conditions..... | 51 |
| 4.3. The intra-cultivar variability on water use efficiency at different water status as a target selection in grapevine: Influence of ambient and genotype | 59 |
| 4.4. Clonal behavior in response to soil water availability in Tempranillo grapevine cv: from plant growth to water use efficiency..... | 65 |
| 4.5 Genotype variations in water use efficiency correspond with photosynthetic traits in Tempranillo grapevine clones | 75 |
| Chapter 5: General discussion | 85 |
| Chapter 6: Conclusions..... | 99 |
| References | 103 |

Symbols and abbreviations list

ABA: Abscisic acid

A_N : Net CO₂ assimilation rate

C^* : leaf area specific capacitance

C_{ft} : relative capacitance at full turgor

C_i : Sub-stomatal CO₂ concentration

$C_{t_{lp}}$: capacitance at turgor loss point

CV: Coefficient of variance

DW: Dry weight

E: Leaf transpiration rate

ET₀: Reference evotranspiration

GDD: Growing degree days

g_m : Meshophyll conductance

g_s : Stomatal conductance

J_{max} : Maximum photosynthetic electron transport rate

LAR: Leaf aparation rate

LER: Leaf expansion rate

LMA: Leaf mass area

MWS: Moderate water stress

PAR: Photosynthetic active radiation

R: Leaf respiration

RW: Rewatering

RWC: Relative water content

s_f : symplasmic water fraction

SGR: Stem growth rate

SWS: Severe water stress

TW: Turgor weight

V_{cmax} : Maximum rates for the carboxylation activity of RuBisCo

VPD: Vapour pressure deficit

WUE: Water use efficiency

WUE_i (A_N / g_s): Intrinsic water use efficiency

WUE_{ins} (A_N / E): Instantaneous water use efficiency

WUE_{wp} : Whole plant water use efficiency

WW: Well-watered

ϵ : bulk modulus of elasticity

π_{tlp} : Osmotic potential at turgor loss point

Ψ : leaf water potential

Ψ_{stem} : Stem water potential

$\delta^{13}\text{C}$: Carbon isotope composition

Resumen

La viticultura es cultivo de alto valor económico propio de zonas áridas y semiáridas, motivo por el cual es especialmente vulnerable a la escasez hídrica. Para mejorar la sostenibilidad del viñedo, una posibilidad es sustituir el material vegetal por otro con una mayor eficiencia en el uso del agua (EUA). Sin embargo, la sustitución de unas variedades por otras encuentra una alta resistencia debido a las particularidades del mercado del vino, por lo que en la presente Tesis se propone el uso de la variabilidad intravarietal, en este caso aplicado al cultivar Tempranillo. De esta forma, se propone la evaluación de diferentes líneas clonales de Tempranillo recogidas en diferentes campos experimentales de La Rioja (España) y Navarra (España). En condiciones de campo se midieron los genotipos para determinar su estatus hídrico (Ψ_h y g_s) y la eficiencia del uso del agua estimada en hoja (EUA_i). Las medidas obtenidas en diferentes campañas y momentos del año permitieron realizar una evaluación precisa de los genotipos estudiados. Paralelamente, se trabajó con una selección de los genotipos evaluados en campo, sembrados en macetas, en el campo experimental de la UIB, con el fin de evaluar su comportamiento en condiciones ambientales homogéneas. Las macetas permitieron tener un mayor control del estatus hídrico de las plantas y una intensificación de las medidas, que incluyeron parámetros de planta entera como la producción de biomasa y el consumo total de agua. Los resultados muestran como es posible realizar una selección clonal por EUA, hayandose diferencias de un 20% entre los genotipos más y menos eficientes. Sin embargo, hay que tener en cuenta que la fuerte variabilidad ambiental requiere de medidas repetidas entre años, con series temporales largas. En líneas generales, los resultados obtenidos en campo y maceta no muestran una coherencia clara, sin embargo, hay ciertos genotipos que muestran alta y baja eficiencia en ambas condiciones. Por último, la evaluación en maceta permitió un estudio más detallado de los mecanismos fisiológicos que determinan una elevada EUA_i . En este caso, una baja tasa de respiración y una alta conductancia del mesófilo fueron los dos mecanismos identificados que supusieron una ventaja en la EUA de dos de los genotipos seleccionados. Como conclusión, los resultados obtenidos en la presente tesis aconsejan la elaboración de programas de selección intravarietal como una potente herramienta para mejorar la sostenibilidad del viñedo.

Summary

Viticulture is a crop with a high economic value, typical of arid and semi-arid zones, which is why it is especially vulnerable to water scarcity. To improve the sustainability of the vineyard, one possibility is to replace the plant material with another with greater water use efficiency (WUE). However, the substitution of some varieties for others finds a high resistance due to the peculiarities of the wine market, so in this Thesis the use of intracultivar variability is proposed, in this case applied to the Tempranillo cultivar. In this way, the evaluation of different Tempranillo clones collected in different experimental fields of La Rioja (Spain) and Navarra (Spain) is proposed. Thus, under field conditions, the genotypes were measured to determine their water status (Ψ_w and g_s) and the estimated leaf water use efficiency (WUE_i). The measurements obtained in different campaigns and times of the year allowed a precise assessment of the genotypes studied. At the same time, a selection of genotypes evaluated in the field were planted in the experimental field of the UIB, in pots, to evaluate their behavior under homogeneous environmental conditions. The pots allowed greater control over the water status and an intensification of the measurements, which included parameters of the whole plant such as biomass production and total water consumption. The results show how it is possible to carry out a clonal selection by WUE, with a 20% difference between the most and least efficient genotypes. However, it must be taken into account that the strong environmental variability requires repeated measurements over the years, with long time series. In general, the results obtained in the field and pot do not show a clear consistency, however, there are certain genotypes that show high and low efficiency in both conditions. Finally, the evaluation in pots allowed a more detailed study of the physiological mechanisms that determine a high WUE_i . In this case, low respiration rate and high mesophyll conductance were the two identified mechanisms that allow a high WUE. In conclusion, the results obtained in this thesis suggest that intracultivar selection programs could be a powerful tool to improve the vineyard sustainability.

Resum

La viticultura és cultiu d'alt valor econòmic propi de zones àrides i semiàrides, motiu pel qual és especialment vulnerable a l'escassetat hídrica. Per a millorar la sostenibilitat de la vinya, una possibilitat és substituir el material vegetal per un altre amb una major eficiència en l'ús de l'aigua (els EUA). No obstant això, la substitució d'unes varietats per unes altres troba una alta resistència a causa de les particularitats del mercat del vi, per la qual cosa en la present Tesis es proposa l'ús de la variabilitat intravarietal, en aquest cas aplicat a la varietat Tempranillo. D'aquesta manera, es proposa l'avaluació de diferents línies clonals Tempranillo recollides en diferents camps experimentals de La Rioja (Espanya) i Navarra (Espanya). En condicions de camp es van mesurar els genotips per a determinar el seu estatus hídric (Ψ_h i g_s) i l'eficiència de l'ús de l'aigua estimada en fulla (EUA_i). Les mesures obtingudes en diferents campanyes i moments de l'any van permetre realitzar una avaluació precisa dels genotips estudiats. Paral·lelament, una selecció dels genotips evaluats en camp es van plantar en el camp experimental de la UIB, en tests, per a avaluar el seu comportament en condicions ambientals homogènies. Els tests van permetre tenir un major control de l'estatus hídric de les plantes i una intensificació de les mesures, que van incloure paràmetres de planta sencera com la producció de biomassa i el consum total d'aigua. Els resultats mostren com és possible realitzar una selecció clonal pels EUA, trobant-se diferències d'un 20% entre els genotips més i menys eficients. No obstant això, cal tenir en compte que la forta variabilitat ambiental requereix de mesures repetides entre anys, amb sèries temporals llargues. En línies generals, els resultats obtinguts en camp i test no mostren una coherència clara, encara que uns certs genotips que mostren alta i baixa eficiència en totes dues condicions. Finalment, l'avaluació en test va permetre un estudi més detallat dels mecanismes fisiològics que determinen una elevada EUA_i . En aquest cas, una baixa taxa de respiració i una alta conductància del mesòfil van ser els dos mecanismes identificats que suposaren un avantatge a l'EUA dels genotips seleccionats. Com a conclusió, els resultats obtinguts en la present tesi aconsellen l'elaboració de programes de selecció intravarietal com una potent eina per a millorar la sostenibilitat de la vinya.

Chapter 1. General introduction

1.- Water availability as a main factor for sustainable viticulture: Opportunities to increase water use efficiency

The water demand for crop production increases each year, and this consumption accounts the main constricting factor for food production (Batchelor et al., 2016). Moreover, the global warming represents an additional threat for the agriculture, with an expected increase of air temperature as well as an increment in the frequency and intensity of climatic anomalies for the near future, such as heat waves and extreme drought episodes (Jones et al. 2010; Hannan et al. 2013; IPCC 2021). Today, climatic change is already happening, and is occurring with a special virulence in semi-arid areas, as Mediterranean region. Moreover, the overall growth of human population induces an increase of food demand so that agriculture enters in competition for land and water use compromising the food production sustainability (Tilman et al., 2011; Ibarrola-Rivas et al., 2017).

Viticulture production, mainly located in semi-arid areas, is seriously affected by global warming, because the grape production and quality are closely linked to climatic conditions (Medrano et al., 2015a; Van Leeuwen et al., 2019). So that, the consequences of the climatic change are already present in most of viticulture regions, with changes in harvest quality due to advance in harvest date, an increment of sugar content and pH levels, or a decrease in total acidity in must (Duchêne and Schneider, 2005; De Orduña, 2010; Mozell et al., 2014).

To compensate water scarcity, Mediterranean vineyard is already being transformed from rainfed to irrigated crop. For example, in the Spain more than 8000 Ha of vineyard each year were transformed during the period between 2014-2019 (Costa et al., 2020). The growing dependency of modern viticulture to water irrigation makes the efficiency of irrigation a main concern for a sustainable viticulture. Consequently, the optimization of water use efficiency (WUE) is a subject of main interest. To improve WUE, is important to monitor the water requirements by plants, avoiding any extra consume. The total water applied can be adjusted, but also the schedule and way to apply water is matter of discussion (Chaves et al., 2010; Wenter et al., 2018; Levin & Kc, 2020; Naulleau et al., 2021). For example, some authors proposed deficit irrigation strategies as regulated deficit irrigation (RDI), partial root-zone irrigation (PRI), (Sadras, 2009; Sepaskhah, & Ahmadi, 2012; Romero et al., 2015;2019; Casassa, et al., 2015; Gil et al., 2018) or direct root zone application (Ma et al., 2020).

There are different strategies to reduce crop water necessities: adapt agronomic practices in order to reduce crop evapo-transpiration, and/or replace genotypes who consume less water.

First, there are methods that can help to reduce water consumption by reducing the whole crop evapotranspiration. For instance, the mulching is used as a protective layer that decrease direct water loss by the soil (López Urrea et al 2012; Fraga et al., 2018). Cover-crop is also used in soil vineyard management because of several benefits. Indeed, the competition for water between the vine and the herbs during the early season will force the vines to reduce their plant vigour. Those vines with less leaf area will consume less water during the summer, so will be better adapted to water stress (Muscas et al., 2017; Linares Torres, et al., 2018). Other practices have been testing the influence of the inter-vines spacing and plant crop density, in vine water consumption (Van Leeuwen et al., 2019). Finally, other technics focused on changing vine stems orientation (Buesa et al 2020a; Bellvert et al., 2021) to change the radiation intercepted by the plant, and thus its capacity to loss water for temperature regulation.

The second general way of adapting viticulture to current changing conditions is to look for varieties and rootstocks with enhanced water use efficiency (OIV, 2019). In this context, the use of improved rootstocks, with more than 80% of worldwide vineyard grafted (Ollat et al., 2016) is plenty of interest. A deeper and denser root system would provide wines an enhanced drought tolerance, thus providing access to increased water resources. It is recognized that rootstocks can influence scion phenotypes in different ways: affecting the rhizosphere interaction, changing the water and the nutrient uptake capacity and differing in the signalling regulation between root and shoot (Gauthier et al., 2020). Despite these advantages, only few rootstocks' genotypes are being used worldwide (Zhang et al., 2016 and the references there in; Gauthier et al., 2020).

The other possibility is the scion replacement. It is widely known that there is a wide variability of genetic resources in grapevine (This et al., 2006; Anderson and Aryal, 2013) with thousands of cultivars with different environmental adaptations. Furthermore, the main commercial cultivars have a wide variation among different commercial clones. The following introduction points will be focused on the scion genetic variability.

2.- Water Use Efficiency: scales of definition and methodologies of measurement

The Water Use Efficiency (WUE) has been largely studied during the history of crop science, and it can be measured at different scales in term of space (from leaf to plant or from plant to crop) as well as in terms of time integration (instantaneous, minutes, day, growing season). Different variables can be used to estimate the Carbon fixation part (instantaneous gas exchange, biomass accumulation, yield, Carbon isotopes), and also the water losses (leaf gas exchange, flow meters, gravimetry). The different ways to calculate WUE depend on the application focus from pure agronomic purposes to detailed physiological studies (Condon et al., 2004; Medrano et al., 2015a).

For a farmer, or wine maker, the most interesting information is based on crop WUE (WUE_c), that accounts for the crop yield divided by the total water applied as irrigation along the growing season. This way to estimate WUE_c integrates total growing season (large time scale), and accounts on one hand, all the water fluxes of the vineyard (precipitation, irrigation and direct losses by soil evaporation and crop transpiration). On the other hand, it accounts only a part of the carbon fluxes (the carbon allocation dedicated for the harvested part of the plant), but is easy to measure and reflects the irrigation water productivity, a parameter of major interest to qualify the agronomic procedures (Chaves et al., 2007; Medrano et al., 2012; Bravdo et al., 2017)

To increase the precision of the water and carbon fluxes measurements several studies have focused on the whole plant WUE. Different technics for the quantification of water consumption and carbon fixation have been previously described. One consists in measuring the water loss of individual pots usually by gravimetry. This way has been widely used to estimated genetic variability of WUE in a lot of different woody crops (Tomas et al., 2012; El Aou-ouad et al., 2018; Buesa et al., 2020b), olive (Bacelar et al., 2007; Ferreira et al., 2018) or prunus (Opazo et al., 2019; Martínez-García et al., 2020). Also at field conditions, with a lysimeter it is possible to quantify with high precision the plant water consume (López-Urrea et al., 2012; Williams et al., 2020) thus enabling a more accurate estimation of the WUE. In this type of experiments the final biomass at the end of the growing season (or fruit production) is used to estimate the carbon accumulation, and is so another long-time integration scale of WUE. Another method consists in isolate a whole plant (in pot or in the field) inside a whole plant gas exchange chamber to measure the whole plant transpiration and photosynthesis. This allows to estimate the instantaneous and the daily photosynthesis and transpiration rates of the plant

and has been tested in wines by different teams, in some cases with controversy on the results (Peña & Tarara, 2004; Poni et al., 2009; Poni et al., 2014; Escalona et al., 2016, Douthe et al., 2018). The experimental set-up is quite complex and the measurements cannot be extended to a wide number of plants simultaneously. The main advantage is the increased resolution of the fluxes estimated, both in terms of space (individual plant is isolated and measured) and time (instantaneous measurements) (Medrano et al., 2010).

Finally, the WUE can be estimated from the fluxes of both carbon and water at the leaf blade. In this case, the carbon gain is reflected by the net assimilation rate (A_N), and the water lost by the transpiration rate (E), both measured with infra-red gas analysers, that gives an instantaneous estimation of instantaneous WUE ($WUE_{ins}=A_N/E$). Because the transpiration rate is strongly influenced by environmental factors as air relative humidity, temperature, and wind velocity, the use of the intrinsic WUE (WUE_i) could provide a more accurate way for comparative purposes among experiments under different environmental conditions (Medrano et al., 2015b). WUE_i refers to the ratio between A_N and stomatal conductance (g_s) to accounts for water loss, instead of the transpiration rate (so $WUE_i = A_N/g_s$). Nevertheless, any change in stomatal conductance will automatically affect both Carbon and water fluxes, the quotient will reflect the efficiency of the system to uptake CO_2 for a determined stomatal aperture. The use of g_s instead of E ensures that the WUE estimated, and its variability, is coming more from the genetic characteristics of the measured plant. This increases comparability between the tested environmental conditions or among the studied genotypes because is theoretically independent of environmental conditions. The availability of precise portable equipment's able to measure both water and carbon fluxes at time and calculate the leaf gas exchange parameters as net photosynthesis and stomatal conductance thus the estimation of WUE_i under realistic measurement conditions. This kind of measurements are being widely used to compare irrigation treatments, agronomic practices or to evaluate large genotype collections (Mathobo et al., 2017; Zufferey et al., 2018; Rodríguez-Gamir et al., 2021). For this reason, in this study, such WUE_i measurements were chosen to compare the genetic variability of WUE within the genotypes of *Vitis vinifera*.

Another method to estimate WUE consists to use a surrogate, that is isotopic composition of the carbon 13 ($\delta^{13}C$) in a given plant tissue. The ^{13}C isotope is a stable C form which is also fixed by the leaves in photosynthesis process. As early shown by Farquhar and colleagues (Farquhar et al., 1980) the atmospheric CO_2 when is fixed by

leaves suffers a discrimination such a down fixation of the existing ^{13}C leading to plants biomass always depleted in ^{13}C compared to the atmospheric ratio of $^{12}\text{C}/^{13}\text{C}$. Any biological process (Calvin Cycle mainly, but also Krebs cycle) is also influencing the ^{13}C discrimination ($\delta^{13}\text{C}$). Farquhar and Richards (1984) identified a very close relationship between WUE and instantaneous discrimination of ^{13}C ($\delta^{13}\text{C}$). This parameter offers the advantage of an integrative evaluation of the WUE all along the time in which the tested biomass sample was assimilated and the concomitant metabolic processes, thus it results in an interesting integrative parameter of the whole WUE. In that way, $\delta^{13}\text{C}$ has been widely used as an integrative indicator of plant water status and plant water use efficiency in grapevine (Santesteban et al., 2015; Bota et al., 2016; Bchir et al., 2016)

3. Grapevine intraspecific variation traits in response to water stress:

As mentioned above, thousands of grapevine cultivars have been described around the world (This et al., 2006; Anderson and Aryal, 2013). Moreover, it is recognised that grapevine is well adapted to semi-arid environments and shows a worldwide distribution (Santillan et al., 2019). The wide diversity of traits which allowed to adapt grapevine to specific environments, including semi-arid ones, as the identification of key parameters that confers tolerance to drought and high temperature is a main goal to understand how grapevine may adapt to climatic change as to choose the most adapted genotypes.

Large genotype variability has been described in responses to drought, regarding leaf photosynthesis, stomatal conductance and water use efficiency (WUE) by different grapevine cultivars (Bota et al., 2001; Costa et al., 2012; Prieto et al., 2010; Soar et al., 2006; Tomàs et al., 2012, Tomàs et al., 2014a). Stomatal control is the key mechanism that regulates the compromise between water loss and CO_2 uptake. Hence, it is a main physiological mechanism modulated in response to water deficit that needs to be studied (Costa et al., 2016). Nevertheless, there is a wide type of responses to water stress and some cultivars seem to have greater robustness in their response type, since they behave in the same way across years and experiments (Bota et al., 2001; 2016; Tomás et al., 2012). This point suggests that the genetic basis of this trait could be stronger in some cultivars than others, which is a hypothesis worth being explored.

Even though several questions about the mechanism of many drought responses still remain unanswered (Gambetta et al., 2020), it is clear that control of water loss by

stomatal regulation is one of the essential points in drought-tolerance strategies (Hochberg et al., 2018).

Abscisic acid (ABA) is involved in stomatal control and probably responsible for the different levels of anisohydry in response to environmental changes. A recent review summarizes current knowledge on the role of ABA in mediating mechanisms responding to abiotic stresses, suggesting to focus future investigation in the basal level of ABA and on the modulations of ABA content in the different grapevine cultivars to characterize abiotic stress tolerance (Marusig & Tombesi, 2020). However, join with the modulation of the hormone control of stomatal aperture, lot of morphologic and physiologic characteristics shows to be also an important player of the whole response to water stress.

The variability among cultivars on water stress response is therefore the result of numerous traits and the links among them and not from the stomatal regulations alone. Variability in xylem architecture has been described between cultivars and related to differences in hydraulic conductivity and responses to water deficit (Hochberg et al., 2015, Dayer et al., 2020). Nevertheless, Alsina et al. (2011), in a study where eight different cultivars were compared, concluded that there was no relationship between embolism vulnerability and leaf drought tolerance traits. More recently, Albuquerque et al. (2020), found similar vulnerabilities to drought-induced xylem embolism in two cultivars (Cabernet Sauvignon and Chardonnay) with different stomatal behavior.

Another player the response to water availability is osmotic adjustment. Several studies have shown differences in this trait in grapevine in response to water stress (Patakas et al., 2003; Martorell et al., 2015a; Düring, 2016; Hochberg et al., 2017), and evidence for differences in osmotic adjustment among cultivars more and less drought adapted has been reported (Martorell et al., 2015b). Levin and Kc (2020) also suggested that differences in osmotic adjustment among cultivars may play primary major role in determining the observed differences in stomatal behaviour of 17 grapevine cultivars. New studies including more cultivars are needed to corroborate this point.

Diffusive limitation to CO₂ under drought is not only imposed by the stomata but also by mesophyll conductance (g_m) (Flexas et al., 2002). Tomas et al. (2014b) found a significant variability of g_m among several grapevine cultivars and associated them with WUE changes. In fact, increases in g_m would lead to improvements of WUE because A_N increases without extra water losses cost thus this parameter seemed to be a promising way to improve the WUE. However, their tedious and complex determination limits seriously their application in selection programs.

Any case, it should be noted that WUE is a complex multi-trait phenotype related with not only stomatal control but also with leaf structure, leaf biochemistry and leaf diffusive properties (Tomás et al., 2014b).

At the biochemical level, plant hormones, secondary metabolites and other key molecules such as carbohydrates, amino acids and polyamines play crucial roles in stress tolerance mechanism. Undoubtedly, all these traits were shown to have high environmental plasticity (Lovisolo et al., 2010; Martorell et al., 2015a; Hochberg et al., 2015, Hochberg et al., 2018). Hence, it must be clarified to what extent differences in water stress tolerance among cultivars result from innate genotypic differences or environmental factors.

4. Intra-cultivar genetic variability in WUE, new opportunities for future breeding programs

Although a high number of cultivars are around the world, only a few elite cultivars occupy the majority of the planted area (Anderson & Aryal, 2013). For example, in Spain, only four cultivars, Airen, Tempranillo, Bobal and Grenache, account more than 60% of total cultivated area (Ibañez et al., 2015). This situation is due multiple factors. One of them is related to the specificity of wine market. Consumers use to prefer the same cultivars, because they relate the name of a given variety with their expectations on wine quality (Eibach & Töpfer, 2015). Secondly, planting a vineyard is a capital consuming operation, because vines will start to produce wine only after three or four years following plantation, but continue to do so for 25-50 years more. For this reason, winegrowers tend to be conservative about the choice of cultivars. Moreover, in most of the highly reputed wine production regions regulation rules include a restrictive list of authorized cultivars.

Cultivated grapevines are vegetatively propagated. As a result, the genome of each cultivar accumulate somatic mutations that resulted in a range of clones from a given cultivar (Ramu et al., 2017; Vondras et al, 2019). Clonal selection programs aim at exploiting this intra-cultivar diversity to improve some agronomic traits.

The origins of clonal selection programs go back to the end of nineteenth century in Germany, and it spread rapidly throughout Europe. The introduction of certified clones to the market was considered being a revolution; vineyards with mixed cultivars and

usually with high levels of viruses were replaced by a single clone field, with virus-free plants, higher uniformity and supposed improved performance (Rühl et al., 2003).

Sometimes clonal selection was a tool for the adaptation of world-famous varieties to the local production environments. In this sense, clonal selection allows to increase the climatic distribution range of one single cultivar, but always under certain limits. In the recent past, most of the vineyard's regions developed public clonal selections programs to standardize different genotype performances in yield or quality parameters, and in parallel, commercial nurseries started to certificate their own genotypes. For example, only in Spain more than 70 certified clones of Tempranillo cultivar are currently available (Ibañez et al., 2015).

In the actual Climate Change context, clonal selection is a possible way to adapt vineyards to new climatic conditions, with a relative easy market acceptability.

The actual success of certified clones, however, implies an increasing genetic erosion because a limited number of clones are replacing old vineyards with great genetic diversity. In an attempt to conserve part of this variability, many public and private institutions are creating accession collections, introducing plants from old or particular vineyards. These collections also allow the characterization of the more interesting accessions for the wine industry. For example, Muñoz et al. (2014) found variability in anthocyanin profiles among Malbec clones, and found some genes related with these differences, and Tello et al. (2018) found differences in pollen viability among Tempranillo clones, that could possibly explain differences in yield and wine composition. Other groups focus on the interaction between climate change and agronomic responses of clones. For example, Arrizabalaga-Arriazu et al. (2020) studied genotypic performance differences between Tempranillo clones grown at high temperatures and high CO₂ concentration and found differences in biomass accumulation and photosynthesis among other characters.

Having in mind the successful of clonal selection programs to adapt some reputed cultivars to specific climatic and/or edaphic conditions, we proposed in this thesis an evaluation of the available genetic diversity to improve WUE inside one specific cultivar.

Tempranillo has been chosen due to its wide distribution around the world, with a huge number of certified clones (Ibañez et al., 2015). Moreover, there are some public

and private clonal collections, with hundreds of Tempranillo clones already characterized in terms of grape production, quality and agronomic performance.

The main tool used to characterize genotypes WUE was the instantaneous measurements of leaf gas exchange to estimate the WUE_i . This method presents the advantage to be rapid and ease of use, allowing to measure a large number of plants. However, because WUE_i is calculated as a ratio of A_N and g_s , all sources of environmental variability affecting these two parameters should be considered (daily circadian cycles, temperature, water stress, light, vapour pressure deficit, leaf age) (Escalona et al., 2003; Prieto et al., 2012; Kaiser et al., 2015; Resco de Dios et al., 2017; Riffle et al., 2021).

The characterization of the WUE variability of Tempranillo clones has been performed in different steps. The first one consisted to make a screening of large number of genotypes under field conditions. From this pool, a selection of genotypes was used to conduct pot experiments to evaluate the response of those genotypes to different degree of soil water availability under controlled conditions, limiting the environmental sources of variation. Finally, a more accurate photosynthetic and hydraulic evaluation were done on six specific genotypes trying to understand the physiological basis of the observed differences in WUE.

Chapter 2. Objectives

2.1. General objectives

As previously presented in the general introduction, the water use efficiency is an important issue for environmental sustainability of crops production. This is especially relevant in the case of the vineyard, due to its distribution in arid and semi-arid areas. Although previous studies shown interesting variability in WUE among cultivars, but the substitution of some reputed cultivars by others more drought tolerant is not practical way. As previously reflected, this can be due multiple factors, as the specificity of wine market. Consumers use to prefer the same cultivars, because they clearly relate the name of a given variety with their expectations on wine quality. Secondly, planting a vineyard is an expensive decision, that will start to produce wine only three or four years later but continue for 25-50 years more. For this reason, winegrowers tend to be conservative about their chosen cultivars. Moreover, in almost of the highly reputed wine production regions there are regulation rules that includes a list of authorized cultivars and avoiding its replacement. For all those concerns, to explore the clonal variability on WUE in a single cultivar, can be a practical way to face drought in a Climate Change context.

In the present Thesis, the main objective was to study the genetic variability for the character water use efficiency inside a wide cultivated grapevine cultivar, Tempranillo, and study the stability of this character among seasons and environments, trying to understand the basis of the observed variability.

This general aim is divided in five specific objectives:

1. To study the genetic variability within the Tempranillo cultivar in relation to the variability of a collection of multiple grapevine cultivars at field conditions.
2. To establish a new method to evaluate genotype water use efficiency performance based in the correlation between WUE_i and g_s to minimize the influence of water status in the evaluation of genotypes.
3. To analyze the interaction of the environment vs. genotype on the WUE variability.
4. To compare the genotypes performance under field and pots conditions.
5. To understand the physiological basis of the variations of water use efficiency in elite clones of Tempranillo.

Chapter 3: Material and methods

3.1 General overview

The present thesis was divided in five experiments with different goals, plant materials, and parameters measured (Fig. 1). First, the WUE_i variability among cultivars and Tempranillo genotypes measured at field conditions and under different plant water status was compared. In the second experiment, we presented field data of WUE_i measured in Tempranillo genotypes, collected during different campaigns in order to determine if this parameter is stable between years and comparable to a whole plant scale evaluation (WUE_{wp}). Third, we compared field and pot growth conditions trying to quantify the environmental influence in photosynthetic traits. Fourth, we made a WUE evaluation at different scales of a selected Tempranillo genotypes in pot conditions during two consecutive campaigns. Finally, we focused on six contrasting genotypes by its WUE trying to explain the physiological basis that underlie the WUE variations.

| | Main question | Plant material | Measured parameters | |
|-------|------------------------------------|---|--|--|
| FIELD | <u>Experiment 1</u> Chapter 4.1 | How much variability on WUE among genotypes of a single cultivar can be? | 30 Tempranillo cv and a 23 cultivar collection | Water status and photosynthetic parameters |
| | <u>Experiment 2</u> Chapter 4.2 | Is it the WUE a stable and valuable criteria to make a selection program? | 14 Tempranillo cv clones measured in two different locations and measured during 3-5 campaigns | Water status, photosynthetic parameters and yield |
| | <u>Experiment 3</u> Chapter 4.3 | Are the field and pots photosynthetic traits comparables? | A firsts comparison between field and pot measurements on the same 7 Tempranillo genotypes | Water status and photosynthetic parameters |
| POTS | <u>Experiment 4</u> Chapter 4.4 | Is the variability in the WUE conserved also under more homogeneous environmental conditions? | A evaluation of 23 Tempranillo genotypes during two consecutive campaigns | Water status, photosynthetic parameters, plant growth and biomass accumulation |
| | <u>Experiment 5</u> Chapter 4.5 | What are the physiological basis of the observed differences? | A nuclear collection of 6 genotypes with differences in its WUE | Water status, photosynthetic parameters, plant growth, biomass accumulation, P-V curves and A_N/C_i curves |

Figure 1. General diagram of the development of the thesis divided into main questions, plant material used and measured parameters in each experiment.

3.2. Plant material

3.2.1. Intercultivar WUE studies

Twenty-three grapevine cultivars were studied during August 2011 in an experimental vineyard located in Palma de Mallorca (39° 35'N, 2°39'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 thereafter rain fed until harvest.

3.2.2. Intraclonal WUE experiments

3.2.2.1. Field experiment

In the first experiment, thirty Tempranillo genotypes 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 (42° 28' N, 2° 27' W). 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 clone's 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 plant water status estimated as water potential or stomatal conductance.

The second experiment presented in these Thesis (point 4.2) has been conducted in two experiment sites, both in Northern Spain. The first one is 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, five clones (232, 807, 1048, 1052, 1084) were measured during five consecutive years. The second site is located at the Roda estate (Bodegas Roda, Haro, La Rioja, Spain), where nine clones (6, 44, 78, 109, 121, 155, 215, 260, 463) were measured during 3 consecutive years. In both sites, plants were grafted onto 110-Richter rootstock, trained as a double cordon in La Grajera, and head-trained bush in Haro.

We characterized each site following their climatic conditions. Data had been collected from the 1st of May to the 1st of October, for the same years as we have been measuring gas exchange. We calculated the Growing Degree Days (in °C day⁻¹) as $T_{\text{mean}} - T_{\text{base}}$ (only positive values) for each day, with $T_{\text{base}} = 10^{\circ}\text{C}$. We also used the accumulated standard evapotranspiration ET_0 (mm), and the accumulated precipitation (mm). All data can be found at www.larioja.org/agricultura/es/informacion-agroclimatica/red-estaciones-agroclimaticas-siar/consulta-personalizada.

In the third experiment presented in this Thesis (4.3), 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). 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). All clones used in the different experiments were grafted onto 110-Richter rootstock, trained as a double cordon and similarly pruned and managed on a standard procedure. Measurements were taken at different moments along the summer in order to sample the plants with a different water status.

3.2.2.2. Experiments in pots

In addition, during the 2017 and 2018 a pot experiment was made. The pot experiment was carried out at the experimental field of University of Balearic Island (UIB).

The plant material used in the fourth experiment (point 4.4) includes 23 Tempranillo genotypes from two origins; a public collection of La Grajera (ICVV, Instituto de las Ciencias del Vino y la Viticultura) (Logroño, La Rioja, Spain), and a private collection of Bodegas Roda (Haro, La Rioja, Spain) (Table 1). These two sites of origin present an experimental field with a high number of Tempranillo genotypes, described above. A first agronomic and genetic approach allowed to reduce the genotypes variability in a nuclear collection. The final selection of the genotypes used in the experiment was done from previous field physiological and agronomical measurements inside these nuclear collections to identify the more contrasting genotypes. In addition, three reputed commercial clones were included in the experiment (RJ43, RJ51, and RJ78). All genotypes were grafted on 110R.

For the last experiment a selection of genotypes described above were used.

3.3. Water relations

3.3.1. Plant water status and irrigation treatments in pot conditions

For pots experiments, the plants grown in 20 L pots, filled with organic substrate and perlite mixture (5:1). It was 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.

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. Ψ_{stem} was measured on one leaf per plant in three to six plants for genotype.

3.3.2. Pressure-volume curves

Pressure-volume (P-V) curves were plotted following the procedures described by Sack & Pasquet-Kok (2011). The leaves on which gas exchange had previously been measured were collected for each genotype, weighed and rehydrated in distilled water for 12 h at 4 °C, and then gradually dried in a well-ventilated room at 25 °C. During the drying process, the leaves were weighed, and water potential was measured using a pressure chamber (Model 1000; PMS Instrument Company, Albany, NY, USA) until a complete P-V curve with at least ten points was established. No ‘plateau effect’ was observed for any sample. Leaf area was determined at the start of the dehydration process and dry mass was determined after at least 72 h at 70 °C. The full turgor and turgor loss points were established by considering the highest R^2 of a linear fit for the linear portion of the $-1/\Psi$ vs. $1-\text{RWC}$ relationship. The following parameters were obtained from the P-V curves: osmotic potential at turgor loss point (π_{tlp}), capacitance at turgor loss point (C_{tlp}), leaf area specific capacitance (C^*_{ft}), symplasmic water fraction (s_f) and bulk modulus of elasticity (ϵ) and relative capacitance at full turgor (C_{ft}) were calculated using standardised major axes (SMA; Sack et al., 2003).

3.3.3. Gas exchange measurements

Leaf net photosynthesis (A_N) and stomatal conductance (g_s) were measured in fully exposed mature leaf (one per plant, $n=5-6$ from the shoot apex) 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 $400 \mu\text{mol } CO_2 \text{ mol}^{-1}$ air, PAR was always above saturation levels ($1200 \mu\text{mol photons } / \text{s}^{-1} \text{ m}^{-2}$) and temperature ranged between 30 and 33°C. 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 for this parameter was noticed demonstrating that there is little affected by environmental conditions, in contrast with the similar check for instantaneous WUE (A_N/E).

To determine the A_N/C_i curves, the gas analyser was equipped with a 2 cm² leaf fluorometer chamber Li-6400-40 (Li-cor Inc.). Determinations were conducted on fully developed leaves at the end of the experiment period. The same procedure was followed for each plant: first, a stabilization period until a steady state of stomatal conductance was reached (typically ~20–30 min) under ambient conditions (CO_2 concentration of $400 \mu\text{mol mol}^{-1}$, PAR of $1500 \mu\text{mol photons m}^{-2}\text{s}^{-1}$, 25 °C). After stabilization, the A_N/C_i curve was generated by changing the concentration of CO_2 entering the leaf chamber in the following steps: 400, 300, 200, 150, 100, 50, 400, 400, 500, 600, 800, 1000, 1200 and $1500 \mu\text{mol } CO_2 \text{ mol air}^{-1}$, with typically 2–3 min between each step. Each A_N/C_i curve was corrected for leaks by following the protocol described by Flexas et al. (2007). All A_N/C_i curve-derived parameters were calculated as described by Bellasio et al. (2016). Leaf respiration (R) was measured after the A_N/C_i curves after covering the plants with opaque plastic for 2 h, using the same chamber and conditions.

3.4. Characterization of the differences in WUE_i

We used three different methods to estimate differences in WUE_i . The first one consists in averaging all the values of a given genotype. However, because of the strong influence of g_s upon WUE_i , we have used the method developed by Tortosa et al. (2016) trying to overcome this effect. This consists in establishing the general relationship between WUE_i ~ g_s (all genotypes), and calculate the residuals of each genotype from the general regression line. Data were linearized using log transformation. This method allows to use any large range of g_s (because of different plant water status) without influencing the

calculated WUE_i . Instead, the clones are compared based on the percentage of deviation from the general regression line ($\text{residual}_{\text{clone}} / \text{predicted}_{\text{clone}}$). We finally used a third approach to study in detail differences between two specific clones. For this, we compared their respective slopes and intercepts on their $WUE_i \sim g_s$ relationship.

3.5. Growth parameters

In the pot experiments, plant leaf number and shoot length were measured in all shoots per genotype at the beginning and the end of each treatment period. Shoot length by the plant was the sum of the individual shoot of each plant. The plant growth rate was estimated using stem growth rate (SGR) and a leaf appearance rate (LAR), parameters obtained from leaf number and shoot length measurements. At the end of each experimental cycle (2017 and 2018), the aerial biomass was weighted. Leaf, shoot, and bunches dry weight per plant were determined separately.

3.6. Yield estimations

To estimate the yield of each of the studied clone at field conditions (data available only at the Roda site, 4.2.), we used the data provided by the company. Those data were based on the average yield of 8-10 vines per a given clone. Then we extrapolate these data to get an estimated yield in $t\ Ha^{-1}$, considering a vine density of ~ 3300 plants Ha^{-1} (1.5×2 m).

3.7. Plant WUE

The whole plant water use efficiency (WUE_{wp}) was calculated as a ratio between plant growth or plant biomass divided by water applied by irrigation. Because the irrigation amount was the same for all genotypes, the WUE_{wp} was only dependent of the growth / biomass.

3.8. Statistical analysis

All statistical analyses were performed using R (Team, 2014). First, a global Two-Ways ANOVA was performed with Genotype \times Years as main effect and their interaction, within each site. Then, we performed separated One-Way ANOVAs, within each year to check in which year the Genotype effect was significant. Similarly, a global Three-Ways ANOVA was performed with Genotype \times Years \times Treatment as main effect and their interaction. When significant, we performed a Post-Hoc test ('agricolae' package, Felipe de Mendiburu, 2015) to determine which were different from each other, and so to

estimate a ranking. We also compared the $WUE_i \sim g_s$ relationship (ANCOVA from the 'car' package (Fox and Weisberg, 2011)) of some specific clones, using the `cld` analysis from the 'emmeans' package (Lenth, 2018). Any differences were accepted with $p\text{-value} < 0.05$.

Chapter 4: Results

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

4.1.1 Climatic conditions

Climatic conditions of the two locations were typical of Mediterranean climate, with high temperatures and irradiance during summer (Table 1). 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 °C difference), but 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 1). 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 ($g_s > 0.150 \text{ mol H}_2\text{O m}^{-2}\text{s}^{-1}$), moderate water stress (g_s between $0.150\text{-}0.075 \text{ mol H}_2\text{O m}^{-2}\text{s}^{-1}$) and severe water stress ($g_s < 0.075 \text{ mol H}_2\text{O m}^{-2}\text{s}^{-1}$). 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 g_s max, and g_s reduction by drought. A strong correlation was evident between decrease of g_s (as percentage) and g_s measured under moderate and severe water stress ($r^2 = 0.88$, $p\text{-value} < 0.0001$)

Table 1. Monthly mean temperature, monthly accumulative rainfall, reference evapotranspiration (ET_o) and irradiance during summer time of 2011 in Palma de Mallorca and summer 2015 in Logroño.

| Location | Month | Mean T ^a (°C) | Rainfall (mm) | ET _o (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 |

4.1.2 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 2) clones and accessions of Tempranillo cultivar (Figure 3). The wide range variation of g_s observed between cultivars (Fig 2A), 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 2B). In contrast, Malvasía, Macabeo, Escursac and Chardonnay showed the highest g_s values and in parallel, the lowest WUE_i .

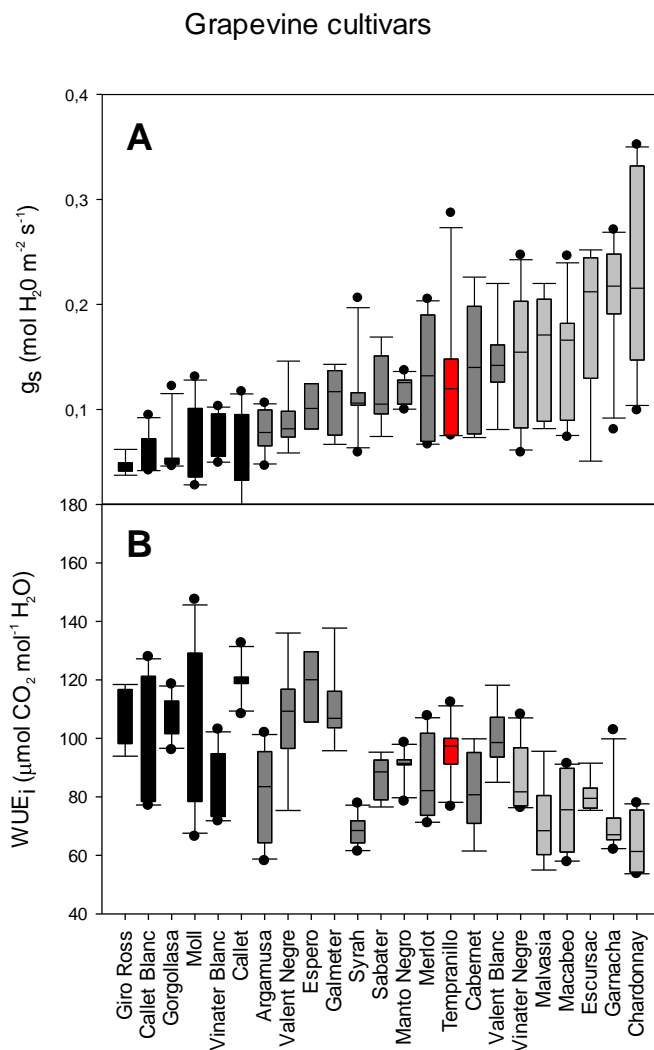


Figure 2. Distribution of stomatal conductance (A) and WUE_i (B) for grapevine cultivars. Black fill: Severe water stress group, dark grey fill; Moderate water-stress and grey fill non-water-stressed group, red fill: Tempranillo grapevine.

Inside cultivar plots, Tempranillo remained in an intermediate position. This cultivar, under moderate water stress conditions showed a WUE_i around $100 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{H}_2\text{O}^{-1}$ at the Majorca experiment (Fig 2B). 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 $\mu\text{mol CO}_2 \text{ mol H}_2\text{O}^{-1}$ (Fig 3B). This range of variation among clones resulted similar to those found among cultivars in the Majorca experiment (118-70 $\mu\text{mol CO}_2 \text{ mol H}_2\text{O}^{-1}$).

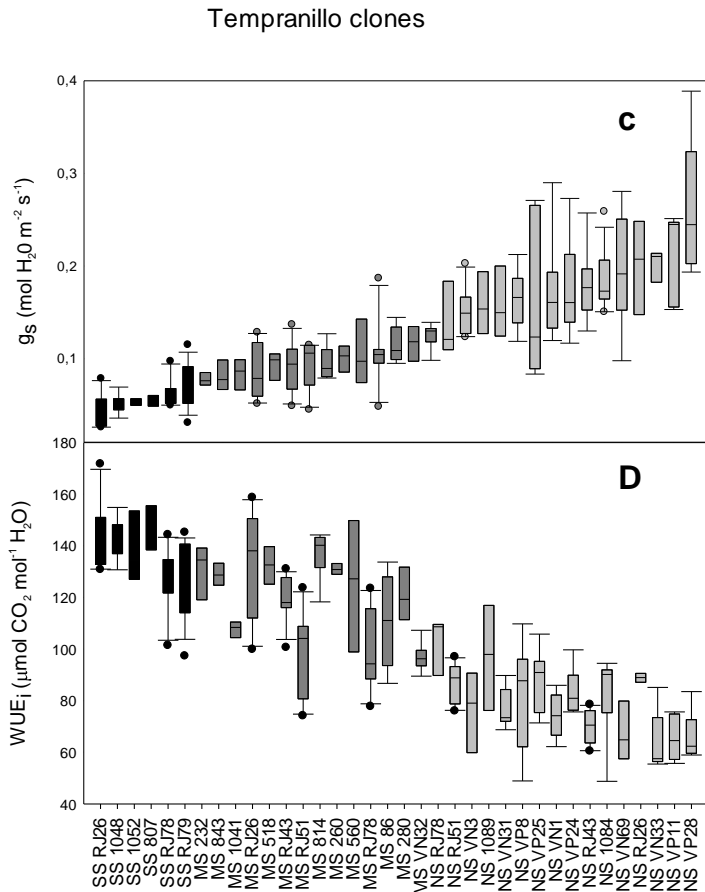


Figure 3. Distribution of stomatal conductance (C) and WUE_i (D) for tempranillo clones. NS: Non water-stress group, MS: Moderate water-stress group, SS: Severe water-stress group. Black fill: Severe water-stress group, dark grey fill: Moderate water-stress and grey non water stress group.

It is highlighted that, for severe water stress, the WUE_i values were higher than for moderate and non-stressed conditions, achieving 120 $\mu\text{mol CO}_2 \text{ mol H}_2\text{O}^{-1}$ for local cultivars in Majorca experiment as Callet (Fig 2B). Inside Tempranillo clones the results showed a WUE_i average of 130 $\mu\text{mol CO}_2 \text{ mol H}_2\text{O}^{-1}$ with the highest values for 807 genotype achieving 147 $\mu\text{mol CO}_2 \text{ mol H}_2\text{O}^{-1}$ (Fig 3B).

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 established as stem water potential (Ψ_{stem}) (Fig 4A) or stomatal conductance (g_s) (Fig 4B). 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 5), 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 4) 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 5).

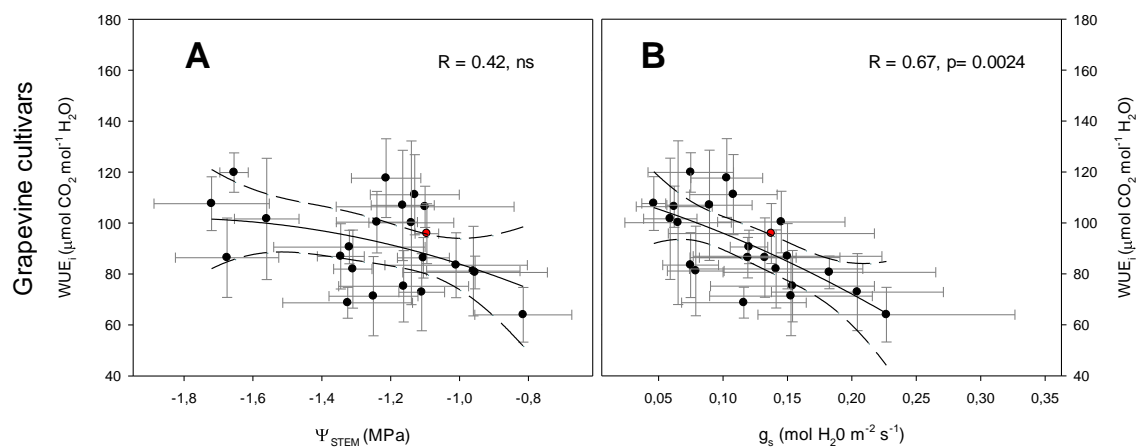


Figure 4. Correlations of WUE_i with leaf water potential (A) and stomatal conductance (B) for grapevine cultivars.

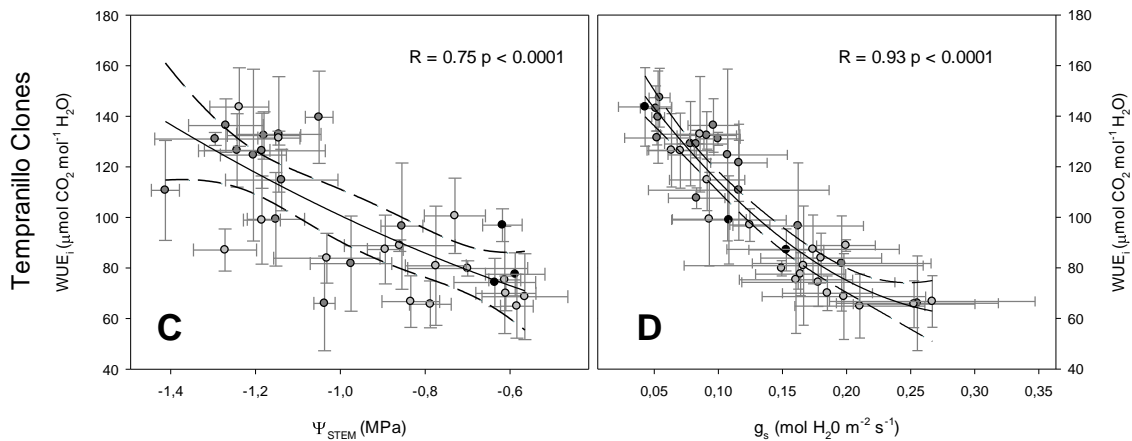


Figure 5. Correlations of WUE_i with leaf water potential (C) and stomatal conductance (D) for tempranillo clones.

These differences between cultivars and clones in g_s and WUE_i average values are more clearly showed in table 2 and table 3 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 2). 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 WUE_i ratios respectively (Table 3). Interestingly, those genotypes also showed an important variability of WUE_i.

Table 2. 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 \pm SD (n=6).

| Group | Cultivar | g_s ($\text{mol m}^{-2} \text{s}^{-1}$) | WUE_i ($\mu\text{mol CO}_2$ $\text{mol}^{-1} \text{H}_2\text{O}$) | Predicted WUE_i ($\mu\text{mol CO}_2$ $\text{mol}^{-1} \text{H}_2\text{O}$) | Predicted WUE_i - Actual WUE_i)/ Predicted WUE_i |
|-----------------|---------------|--|---|--|--|
| Severe stress | Callet | 0.075 ± 0.033 | 119.8 ± 7.7 | 100.8 | 18.8% |
| | Gorgollasa | 0.062 ± 0.029 | 106.4 ± 8.1 | 103.2 | 3.1% |
| | Giro ross | 0.046 ± 0.009 | 107.6 ± 10.6 | 105.9 | 1.6% |
| | Callet Blanc | 0.059 ± 0.021 | 101.6 ± 23.8 | 103.7 | -2.0% |
| | Moll | 0.065 ± 0.041 | 100.1 ± 32.1 | 102.6 | -2.4% |
| | Vinater Blanc | 0.075 ± 0.022 | 83.5 ± 12.7 | 100.8 | -17.2% |
| Moderate stress | Espero | 0.103 ± 0.028 | 117.6 ± 15.5 | 95.3 | 23.4% |
| | Galmeter | 0.108 ± 0.034 | 111.1 ± 15.7 | 94.2 | 17.9% |
| | Valent Blanc | 0.145 ± 0.049 | 100.4 ± 12.1 | 86.0 | 16.7% |
| | Valent Negre | 0.089 ± 0.033 | 106.9 ± 21.6 | 98.0 | 9.1% |
| | Tempranillo | 0.138 ± 0.080 | 95.8 ± 11.8 | 87.8 | 9.1% |
| | 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 | -2.8% |
| | Cabernet | 0.141 ± 0.067 | 82.0 ± 15.3 | 86.9 | -5.6% |
| | Sabater | 0.119 ± 0.038 | 86.4 ± 7.9 | 91.8 | -5.9% |
| | Argamusa | 0.079 ± 0.022 | 81.1 ± 17.6 | 100.1 | -19.0% |
| | Syraz | 0.116 ± 0.048 | 68.7 ± 6.0 | 92.5 | -25.7% |
| No stress | Escursac | 0.183 ± 0.083 | 80.6 ± 6.4 | 76.7 | 5.1% |
| | Vinater Negre | 0.150 ± 0.073 | 86.9 ± 12.8 | 84.8 | 2.5% |
| | Garnacha | 0.204 ± 0.067 | 72.8 ± 15.1 | 70.9 | 2.7% |
| | Chardonnay | 0.227 ± 0.100 | 64.0 ± 10.7 | 64.5 | -0.8% |

| | | | | | |
|--|----------|---------------|-------------|------|--------|
| | Macabeo | 0.154 ± 0.064 | 75.2 ± 14.0 | 83.9 | -10.4% |
| | Malvasia | 0.153 ± 0.063 | 71.3 ± 15.6 | 84.2 | -15.3% |

Table 3. 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 (μ mol CO ₂ mol ⁻¹ H ₂ O) | Predicted WUE_i (μ mol CO ₂ mol ⁻¹ H ₂ O) | (Predicted WUE_i - Actual WUE_i)/ Predicted WUE_i |
|-----------------|--------------------|---|--|--|---|
| 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% | |

4.1.3 Genetic variability of WUE

Coefficient of variance is commonly reputed as good estimator of variability for different characters. Table 4 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.

Table 4. 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 ($\text{mol}^{-1} \text{H}_2\text{O m}^{-2} \text{s}^{-1}$) | | | WUE_i ($\mu\text{mol CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$) | | |
|----------------------------|--|------|-----|--|-------|-----|
| | 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% |

4.2. Variability in water use efficiency of grapevine Tempranillo clones and stability over years at field conditions

4.2.1 Experimental fields comparison and year effect

We compared the WUE of different Tempranillo clones in two experimental sites located in La Rioja (Spain); one located in Logroño belonging to the ICVV Research Institut (“La Grajera” experimental field), and a second one in Haro belonging to the commercial winery Roda. These two locations have a typical Mediterranean climate, with high temperatures and low precipitation in summer. However, slight differences were observed between experimental years and sites (Table 5). The growing degree days were always higher (almost 15%) in La Grajera than in Roda. Related to this, the accumulated ETo is likewise higher and the total rainfall is slightly lower in La Grajera than in Roda.

Table 5. Monthly averages of g_s and WUE_i in the two experimental fields.

| Year | La Grajera | | Roda | |
|------|--|--|--|--|
| | g_s (mol H ₂ O m ⁻² s ⁻¹) | WUE_{int} (μ mol CO ₂ mol ⁻¹ H ₂ O) | g_s (mol H ₂ O m ⁻² s ⁻¹) | WUE_{int} (μ mol CO ₂ mol ⁻¹ H ₂ O) |
| 2015 | 0.09 ± 0.015b | 123.6 ± 6.2a | | |
| 2016 | 0.130 ± 0.012a | 98.1 ± 3.9c | 0.393 ± 0.014a | 51.0 ± 1.5c |
| 2017 | 0.09 ± 0.004b | 99.1 ± 1.8c | 0.132 ± 0.007c | 86.2 ± 1.8a |
| 2018 | 0.082 ± 0.006b | 103.5 ± 2.9bc | | |
| 2019 | 0.084 ± 0.007b | 115.3 ± 2.7ab | 0.303 ± 0.014b | 67.2 ± 2.1b |

Two-Way ANOVA: Year*** Field*** Year x Field***

* Different letters indicate statistical differences within each field by Tukey test (P<0.05)

The water plant status, main determinat of WUE, was indirectly estimated by the stomatal conductance following Medrano and Flexas 2002. To compare the impact of the effect of climatic conditions on plant water status, all years and genotypes g_s values were averaged (Table 5). In La Grajera, g_s varied in average between 0.08 and 0.09 mol H₂O m⁻² s⁻¹ in four over the five studied years, showing values typical of moderate to severe water stress . The year 2016 showed the largest g_s values reaching 0.13 mol H₂O m⁻² s⁻¹. In the case of WUE_i , the range of variation was between 98 and 124 μ mol CO₂ mol⁻¹ H₂O. Interestingly, we noticed a remarkable increase of WUE_i in 2015 compared to 2017 (+25% higher), but being at similar g_s values (average 0.09 mol H₂O m⁻² s⁻¹). In parallel we noted an increased net assimilation rate (A_N) in 2015 compared with 2017 (9.6 and

8.6 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, respectively). The same effect was found when compared 2018 and 2019.

In Roda field, the g_s values were clearly higher than in La Grajera, ranging between 0.13 and 0.39 $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$, that means to be between a mild to moderate water stress. The corresponding range of variation in WUE_i in this site resulted lower than in La Grajera ($p < 0.001$, Table 5) and was between 51 and 86 $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$. lower than

No differences were found when we compared the general slope of the $\text{WUE}_i \sim g_s$ relationship of the two experimental fields (Figure 6). When data of all years were grouped, and when the comparison were done in a specific g_s range, differences were not found between 0.150 and 0.225 $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$, meanwhile La Grajera had higher WUE_i at low g_s , and the reverse at high g_s .

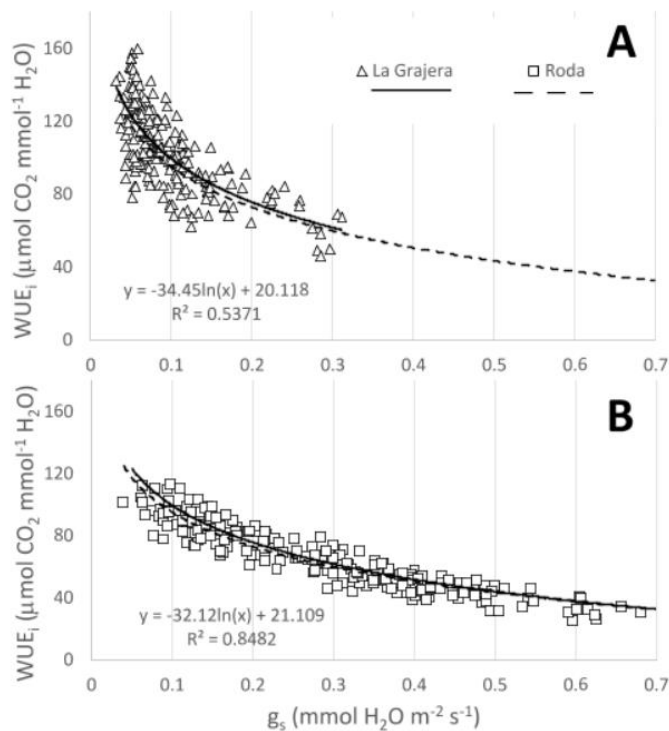


Figure 6. Correlations between WUE_i and stomatal conductance (g_s) in the two experimental sites; La Grajera (A) and Roda (B). Continuous line shows the relation for the La Grajera genotypes and dashed lines shows the same relation for Roda genotypes.

4.2.2 Genotypic variability of WUE_i and stability over years

We found significant Genotype and Year effects (and their interaction) in both La Grajera and Roda sites ($p < 0.001$ in both, Tables 6a and 6b, respectively). In La Grajera, extreme values were reached by clones 1084 and 807 (with 87.7 and 108.5 $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$,

respectively, all year confounded). When each year was analysed separately, the genotype effect was significant within each year in Roda, and in 3 out of 5 years in La Grajera. Moreover, some repetitive pattern was present, like that of the clone 1084, showing systematically the lowest values of WUE_i . A systematic genotype effect within each year was also present in Roda, with some clones (260, 109) showing the lowest values ($\sim 55 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$) and others showing repetitively the highest WUE_i values (clones 463, 44, 6) around $75 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$.

Table 6a. Variation in WUE_i ($\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$) per genotype and year in La Grajera field (values are means \pm SE)

| Genotype | 2015 | 2016 | 2017 | 2018 | 2019 | Average |
|-------------|-------------------|-----------------|-----------------|-------------------|-------------------|------------------|
| 232 | 123.7 \pm 9.4a | 113.8 \pm 8.3 | 106.4 \pm 4.9 | 95 \pm 9.2b | 116.4 \pm 2.8b | 110.9 \pm 3.9a |
| 807 | 129.4 \pm 13.4a | 102.1 \pm 8.1 | 97.4 \pm 2.7 | 122.2 \pm 3.9a | 122.6 \pm 3.6ab | 108.5 \pm 2.6a |
| 1048 | 143.1 \pm 4.5a | 90.9 \pm 7.8 | 101.3 \pm 3.1 | 105.9 \pm 3.1ab | 128.4 \pm 2.9a | 107.5 \pm 3.4a |
| 1052 | 139.6 \pm 12.9a | 94.7 \pm 5.7 | 97.7 \pm 2.7 | 102.7 \pm 1.4b | 113.2 \pm 3.9b | 103.3 \pm 3.6a |
| 1084 | 79.6 \pm 12.1b | 81.5 \pm 9.2 | 92.1 \pm 4.8 | 91.8 \pm 9.8b | 93.1 \pm 2.4c | 87.7 \pm 3.6b |

Two-Way ANOVA: Year*** Genotype*** Year x Genotype***

*Different letters indicate statistical differences within each year by Tukey test ($P < 0.05$)

Table 6b. Variation in WUE_i ($\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$) per genotype and year in Roda field (values are means \pm SE)

| Genotype | 2016 | 2017 | 2019 | Average |
|------------|------------------|-------------------|-------------------|-----------------------------|
| 6 | 56.7 \pm 4.5ab | 96.6 \pm 2.7a | 83.7 \pm 7.1a | 75.1 \pm 4.6ab |
| 44 | 58.5 \pm 4.7ab | 90.6 \pm 6.6ab | 71.8 \pm 6.9abc | 70 \pm 4.2abc |
| 78 | 56.8 \pm 5.7ab | 76.2 \pm 4.8bc | 64.6 \pm 1.5bcd | 64 \pm 3abc |
| 109 | 44 \pm 4.4abc | 76.4 \pm 4.2bc | 48.9 \pm 1.2d | 53.5 \pm 3.3c |
| 121 | 49.9 \pm 3abc | 98.3 \pm 4.2a | 88.4 \pm 2.8a | 78.1 \pm 4.4 ^a |
| 155 | 61.2 \pm 3.1a | 69.5 \pm 3.2c | 57.3 \pm 2.5cd | 62 \pm 1.9abc |
| 215 | 43.3 \pm 1bc | 86.6 \pm 2.3abc | 52.4 \pm 3.4d | 59.3 \pm 4.2bc |
| 260 | 34 \pm 2c | 81.9 \pm 2.6abc | 50.6 \pm 1.9d | 52.8 \pm 4.4c |
| 463 | 55 \pm 5ab | 95.6 \pm 6a | 76.1 \pm 3.8ab | 74 \pm 4.4ab |

Two-Way ANOVA: Year*** Genotype*** Year x Genotype***

* Different letters indicate statistical differences within each year by Tukey test ($P < 0.05$)

To overcome the WUE_i variability induced by the range of variation of g_s , we have also characterised each genotype following its residuals of a general $WUE_i \sim g_s$ relationship (See Introduction and M&M), expressed as percentage (Tables 7a and 7b). Thus, by doing

so, we found globally the same pattern as the previous comparison (see above). In this case, Roda clones showed more variability between them with a significant genotype effect in the three measured years in comparison with only 2 out of 5 measured years in La Grajera. In Roda, the same genotypes were identified as less (clones 260, 215, 109) or more (44, 463) efficient in terms of WUE_i . Moreover, some genotypes were more constant through years than others. We estimated a Year effect for each clone separately, and the clones 260 and 463 (two extremes) were seen as the most constants over the years (no Year effect).

Table 7a. Variation in percentage respect to *predicted value* per genotype and year in La Grajera field.

| Genotype | 2015 | 2016 | 2017 | 2018 | 2019 | Average |
|----------|-------|-------|-------|----------|----------|-------------|
| 232 | 1.7% | 2.1% | 0.5% | -16.2% c | 6.5% a | 0.3% ± 2.4 |
| 807 | -4.3% | -3.7% | -1.9% | 17.4% a | 0.5% a | 0.1% ± 2.3* |
| 1048 | 2.5% | 1.7% | 0.0% | -0.8% b | 2.2% a | 1.1% ± 2 |
| 1052 | 1.7% | 0.6% | 1.4% | 0.1% b | -1.8% ab | 0.4% ± 1.7 |
| 1084 | -2.1% | 2.1% | 0.7% | -3.8% bc | -8.2% b | -0.9% ± 2.1 |

Two-Way ANOVA: Genotype*** Year x Genotype***

* Different letters indicate statistical differences within each year by Tukey test ($P < 0.05$) **Asterisk mean significate differences between year for each genotype

Table 7b. Variation in percentage respect to *predicted value* per genotype and year in Roda field.

| Genotype | 2016 | 2017 | 2019 | Average |
|----------|-----------|----------|-----------|------------------|
| 6 | 2.2% abcd | 8.1% a | -1.8% bc | 2.2% ± 1.9ab |
| 44 | 13.5% a | 10.7% a | -4.0% bc | 8.6% ± 2.5a* |
| 78 | -4.3% bcd | -1.7% ab | 0.7% abc | -1.5% ± 1.7bc |
| 109 | -4.8% cd | -10.2% b | 6.5% a | -1.9% ± 2.0bc*** |
| 121 | -2.7% bcd | 0.0% ab | 4.7% ab | 0.3% ± 1.7bc |
| 155 | 9.5% ab | 0.3% ab | -0.5% abc | 4.0% ± 1.6ab** |
| 215 | -9.2% d | -3.1% ab | -6.1% c | -6.2% ± 1.2c · |
| 260 | -7.1% d | -4.9% ab | -6.5% c | -6.1% ± 1.6c |
| 463 | 8.0% abc | 7.2% a | 2.3% bc | 4.6% ± 2.1ab · |

Two-Way ANOVA: Genotype*** Year x Genotype***

* Different letters indicate statistical differences within each year by Tukey test ($P < 0.05$) **Asterisk or point mean significate differences between year for each genotype (· $p < 0.1$ * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$)

We finally tested more in detail the obtained differences between 2 extreme clones from Roda (260, less efficient and 463, more efficient) (Figure 7). These clones revealed to have different $WUE_i \sim g_s$ relationships, with similar slopes but a higher intercept for 463.

The latter presents a constant higher WUE_i of 10%, over the whole range of g_s compared to 260. Those data were based on all years confounded.

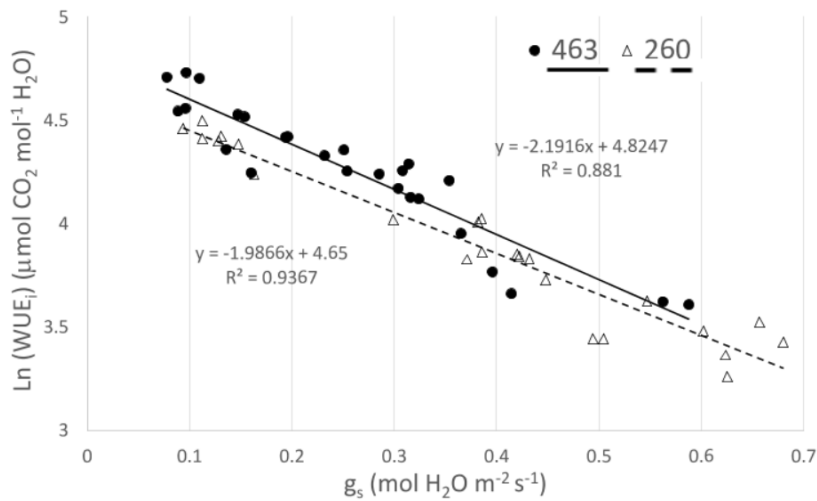


Figure 7. Relationship between the natural logarithm of the WUE_i against g_s , all years confounded, in the two more contrasting genotypes.

4.2.3 Yield variations between clones and over years

Total production variation between clones is here reported for Roda clones (Table 8). This variation is also shown when compared total production within different years. Unfortunately, yield data were not compiled during the same years in which gas exchange measurements were performed. However, we used 7 consecutive years (from 2003 to 2009) from the same experimental site of Roda to estimate the variability in yield of the same 9 tested clones, thus avoiding the potential effect of differential experimental conditions. From the collected data by the company, a huge variability in total production was present between clones, varying from 1.3 to 13.3 t Ha⁻¹ (all years and clones confounded). Because only one data was available per each Genotype per Year combination, we performed separated Anovas. The Genotype effect was significant ($p < 0.001$) opposite to the Year effect. The total production varied from 9.7 to 3.3t Ha⁻¹ for clones 463 and 155, respectively. However, we did not find any clear relationship between yield and WUE_i . Nevertheless, we identified the clone 463 the most productive in terms of yield and one of the more efficient in terms of WUE_i .

Table 8. Production (t Ha⁻¹) of Roda genotypes each year.

| Genotype | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | Gen. av. |
|-----------------|-----------|---------|-------|-----------|-----------|-----------|-----------|-------------|
| 6 | 5.0 | 8.0 | 8.3 | 10.7 | 4.7 | 8.7 | 3.7 | 7 ± 1ab |
| 44 | 4.0 | 3.7 | 2.7 | 4.7 | 2.0 | 3.3 | | 3.4 ± 0.4c |
| 78 | 7.7 | 8.0 | 9.3 | 10.7 | 8.7 | 7.3 | 9.4 | 8.7 ± 0.5a |
| 109 | 7.3 | 7.0 | 8.0 | 13.3 | 8.0 | 7.7 | 10.5 | 8.8 ± 0.9a |
| 121 | 5.0 | 4.3 | 4.7 | 5.3 | 3.3 | 4.0 | 4.4 | 4.4 ± 0.3bc |
| 155 | 5.7 | 4.0 | 1.3 | 2.0 | 2.7 | 3.3 | 3.8 | 3.3 ± 0.6c |
| 215 | 6.7 | 4.3 | 3.3 | 6.7 | 2.0 | 4.7 | 5.5 | 4.7 ± 0.7bc |
| 260 | 7.7 | 3.7 | 4.0 | 7.3 | 1.3 | 6.0 | 8.6 | 5.5 ± 1.1bc |
| 463 | | | 7.0 | 11.3 | 10.0 | 9.3 | 10.8 | 9.7 ± 0.8a |
| Year av. | 6.3 ± 0.5 | 5 ± 0.7 | 5 ± 1 | 7.7 ± 1.3 | 4.8 ± 1.2 | 5.7 ± 0.8 | 7.6 ± 1.1 | |

* Different letters indicate statistical differences within each genotype by Tukey test (P<0.05)

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

4.3.1 Plant water status, environmental conditions and WUE

Considering all the genotypes measured under all experimental conditions, the g_s values ranged between 0.05 and 0.45 mol H₂O m⁻² s⁻¹, showing a large difference in plant water status. The values of net CO₂ assimilations (A_n) ranged from 3 to 21 μmol CO₂ m⁻² s⁻¹, which resulted in a large variability of WUE_i, ranging from 20 to 160 μmol CO₂ mol⁻¹ H₂O. The WUE_i was strongly and negatively related to g_s , as shown in Fig. 8A ($R^2=0.75$). 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 CO₂ mol⁻¹ H₂O, respectively. The regression between WUE_i – g_s for each water status (Fig.8B), 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 non-stressed plants.

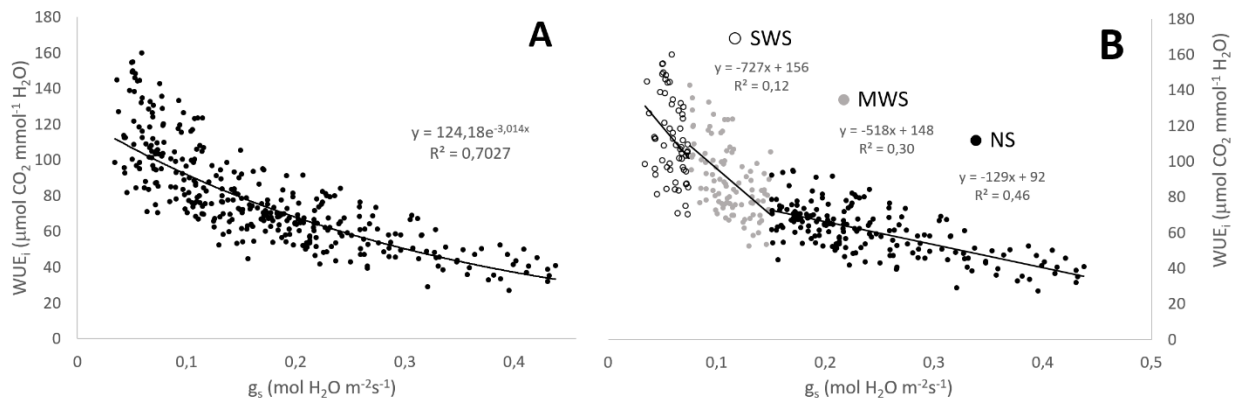


Figure 8.. General correspondence between stomatal conductance (g_s) and intrinsic water use efficiency (WUE_i) (A) and individual relationship between both variables for each water status interval, WW: Well-watered, MWS: Moderate water stress, SWS: Severe water stress (B). Data are all the replicates, grown at field and in pot conditions.

4.3.2 Comparison between pot and field conditions

Under field conditions, the maximum g_s was 0.3, while in pots it was 0.45 mol H₂O m⁻² s⁻¹ (Figure 9A) The WUE_i – g_s relationship showed interesting differences between pots and field conditions. To confirm this observation, we linearized the regressions using the natural logarithm (Fig. 9B). 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 H₂O 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 H₂O m⁻² s⁻¹ this difference were around 10%.

Thus, for similar conditions of water stress (estimated with g_s), the WUE_i was clearly higher for field growing plants.

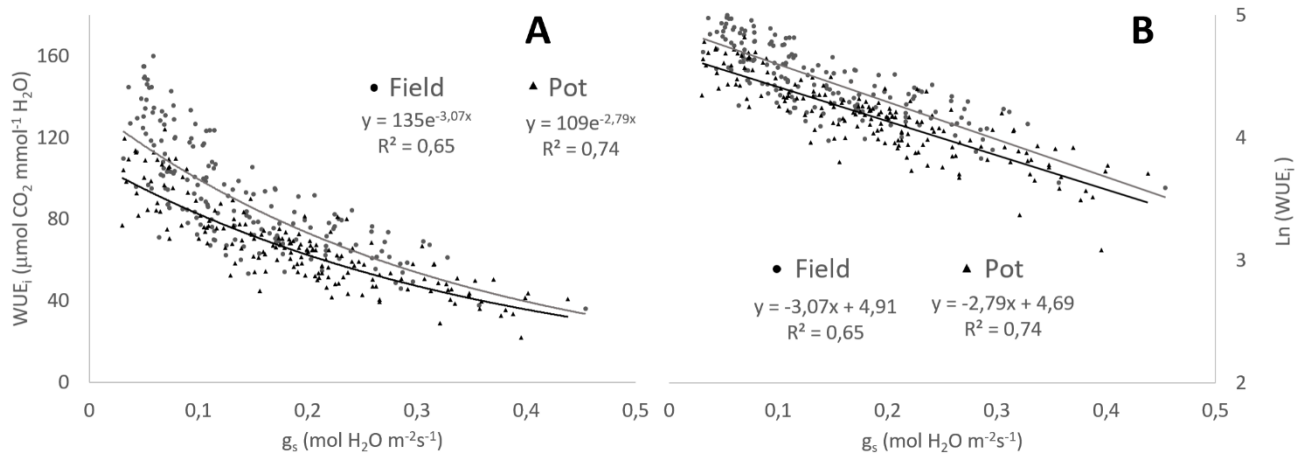


Figure 9. Comparison between field (circles) and pot (triangles) conditions of g_s - WUE_i relationship (A) and linearized regressions using the natural logarithm of WUE_i in order to make an statistical comparison between the genotypes performance (B).

4.3.3 Genotype effect on WUE

To compare the genotypes individual response, a linear logarithm regression of each genotype for the different g_s was done. In all cases, these regressions showed a high and significant degree of correlation, allowing comparing them with an ANCOVA (Table 9).

Table 9. 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 ($\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$) | Field WUE_i ($\mu\text{mol CO}_2 \text{ mmol}^{-1}\text{H}_2\text{O}$) | Pot WUE_i ($\mu\text{mol CO}_2 \text{ mmol}^{-1}\text{H}_2\text{O}$) | Difference |
|--|---|---|------------|
| 0,075 | $107,5 \pm 1,02$ | $86,9 \pm 1,02$ | 19% *** |
| 0,15 | $85,8 \pm 1,01$ | $71,3 \pm 1,01$ | 17% *** |
| 0,3 | $54,6 \pm 1,03$ | $48,1 \pm 1,02$ | 12% *** |

*** indicates significant differences between points (p -value <0.001)

Comparing the performance of each individual genotype under pot and field conditions, (Table 10) the genotype 232 showed the highest negative slope. Under field conditions, the $WUE_i - g_s$ showed R^2 between 0.25 and 0.73 (average = 47.7) while in pots conditions they varied between 0.48 and 0.85 (average = 75.1). 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.

Table 10. Pearson coefficient (R^2 and slopes) of the g_s - 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.

| Genotype | Field conditions | | Pot conditions | | Field+pot | |
|----------|------------------|------------------|----------------|--------------------------------|-----------|--------------------------------|
| | R^2 | Slope | R^2 | Slope | R^2 | Slope |
| 232 | 0,25* | -4,28 \pm 1,62 | 0,72*** | -2,40 \pm 0,27 ^{ab} | 0,71*** | -3,40 \pm 0,30 ^{ab} |
| 1048 | 0,58*** | -2,62 \pm 0,47 | 0,76*** | -3,10 \pm 0,33 ^a | 0,65*** | -3,16 \pm 0,32 ^{ab} |
| 1052 | 0,52*** | -3,13 \pm 0,75 | 0,88*** | -3,68 \pm 0,34 ^{ab} | 0,79*** | -4,03 \pm 0,35 ^a |
| 1084 | 0,73*** | -2,95 \pm 0,4 | 0,78*** | -3,00 \pm 0,41 ^{ab} | 0,67*** | -2,96 \pm 0,34 ^{ab} |
| RJ43 | 0,6*** | -2,68 \pm 0,37 | 0,79*** | -2,98 \pm 0,35 ^{ab} | 0,71*** | -3,16 \pm 0,27 ^{ab} |
| RJ51 | 0,62*** | -2,73 \pm 0,44 | 0,48*** | -2,18 \pm 0,57 ^{ab} | 0,55*** | -2,50 \pm 0,35 ^{ab} |
| RJ78 | 0,58*** | -3,02 \pm 0,49 | 0,85*** | -2,26 \pm 0,16 ^b | 0,79*** | -2,71 \pm 0,18 ^b |

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Under pot conditions, 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 $0.45 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$. The resultant slopes varied with the same amplitude than in field conditions, ranging between -3.7 to -2.2 (Table 10). In this case, the interaction factor of the ANOVA was significant (p -value < 0.01). This interaction was due to a significant difference between genotypes 1052 and RJ78. 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. 10). At low water availability the 1052 showed higher WUE_i than the RJ58, while at higher g_s the opposite was observed (Fig. 10 and Table 11).

Table 11. Comparison of genotypes 1052 and RJ78 in WUE_i calculated by the natural logarithm regression at different g_s values.

| g_s ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) | 1052 $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$ | RJ78 $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$ | Difference |
|--|--|--|------------|
| 0,05 | 115,3 \pm 1,04 | 104,3 \pm 1,04 | 10% . |
| 0,1 | 94,3 \pm 1,03 | 91,1 \pm 1,03 | 3% |
| 0,2 | 63,1 \pm 1,04 | 69,4 \pm 1,02 | 10% ** |
| 0,3 | 42,2 \pm 1,06 | 52,9 \pm 1,03 | 26% *** |

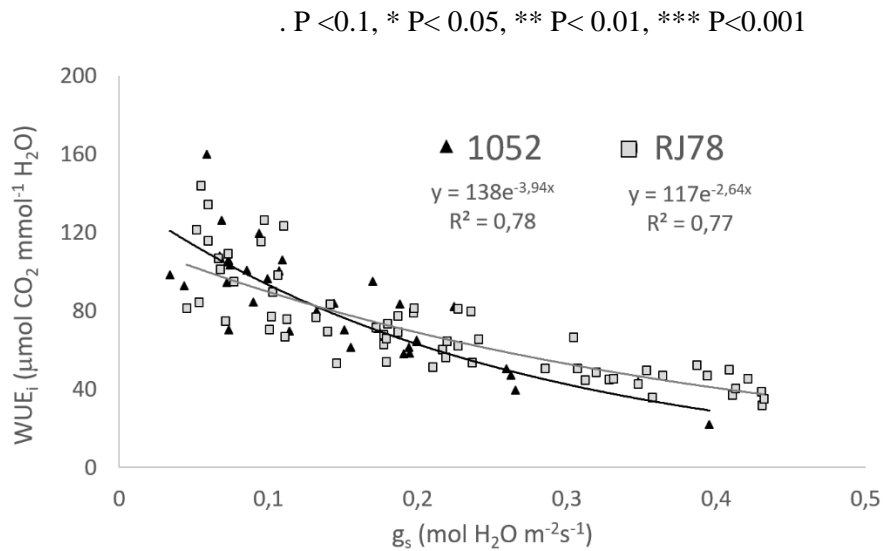


Figure 10. Comparison of genotypes RJ78 (grey squares) and 1052 (black triangles) in WUE_i slopes considering the whole range of stomatal conductance combining field and pot data (FIELD+POT).

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 slope comparison between genotypes but grouping field and pot data of each of them, and the 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 10) which showed to be highest without water stress (g_s higher than $0.2 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) (Table 11). Then, genotype 1052 was clearly more conservative in the use of water under non-stressed conditions.

4.4. Clonal behavior in response to soil water availability in Tempranillo grapevine cv: from plant growth to water use efficiency

4.4.1. Plant growth

Under well-watered condition, the stem growth rate (SGR) was considerably higher in the first year of study compared to the second year (Fig 11A-B), showing mean values of 4.4 and 2.2 cm day⁻¹ respectively. The first year of study, the genotypes 215 and 232 showed the higher SGR (5.9 and 5.6 cm day⁻¹) and genotypes 336 and 1052 the lowest (2.7 and 3.5 cm day⁻¹) (Fig 11A). In the second year of study, genotype 243 presented the highest SGR (3.4 cm day⁻¹ and) against genotype 108 (1.6 cm day⁻¹) (Fig 11B).

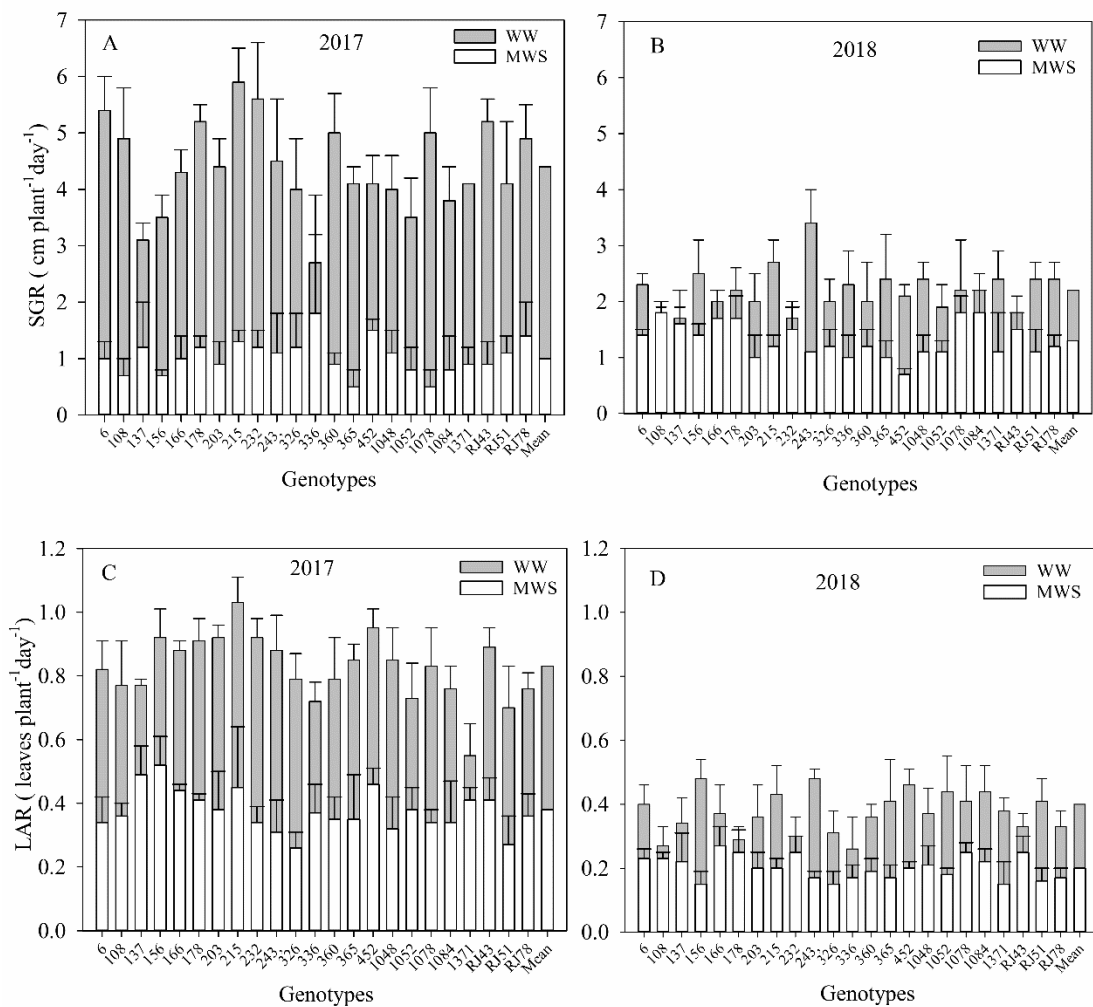


Figure 11. Stem growth rate (SGW) and leaf appearance rate (LAR) of each genotype measured in 2017 (A, C) and 2018 (B, D) in well-watered (WW) (grey bars) and moderate water stressed (MWS) (white bars). Values are the mean \pm SE (N=5).

Leaf appearance rate (LAR) also was similarly affected by the year as SGR. Under well-watered condition (WW), plants during 2017 showed a mean of LAR twice than during 2018 (Fig 11C-D). These differences between years were likely due to the presence of bunches in 2018 that implied a reduction in vegetative growth represented by the two

studied parameters. In 2017, genotype 215 showed higher LAR than RJ51 (1.03 leaf day⁻¹ and 0.55 leaf day⁻¹, respectively). In 2018, genotype 243 had the higher LAR and genotype 108 the lowest LAR (0.48 and 0.27 leaf day⁻¹ respectively).

In both years, moderate water stress (MWS) significantly reduced SGR ranged between 25 and 90% (Fig 11A-B). This reduction was in general higher during 2017, likely due to a higher SGR. MWS condition also affected LAR, showing a reduction between 54 and 44% respect to WW condition for 2017 and 2018, respectively. Under this condition, the variability between genotypes in both parameters was lower than under WW condition, especially during 2018. Then, in 2017, the genotype 452 showed the higher SGR and LAR, (1,5 cm day⁻¹ and 0.46 leaf day⁻¹ respectively), and the genotype 1078 showed the lowest values of these two parameters (0.5 cm day⁻¹ and 0.34 leaf day⁻¹, respectively). On the contrary, during 2018, genotype 452 presented the lowest SGR (0.7 cm day⁻¹) and genotype 1078 the highest (1.8 cm day⁻¹).

Statistical analysis showed that the genotype effect was significant for the LAR ($p < 0.05$), but not for SGR considering both years of study (Table 12).

Table 12. Three-Ways ANOVA performed for Genotype \times Years \times Treatment as main effect on Growth, Biomass and gas exchange parameters, and their interaction.

| Effects | Growth | | Biomass | | | | Gas exchange | | |
|--------------------|--------|-----|---------|--------|---------|-------|--------------|-----|------|
| | SGR | LAR | Leaves | Shoots | Bunches | Total | gs | A | WUEi |
| Genotype (Gen) | | * | *** | *** | | * | *** | * | |
| Year | *** | *** | ** | *** | - | *** | | | ** |
| Trat | *** | *** | - | - | - | | *** | *** | *** |
| Gen x year | | | ** | | - | - | | | |
| Gen x Treat | | | - | - | - | . | | | |
| Year x Treat | *** | *** | - | - | - | . | *** | *** | *** |
| Gen x Year x Treat | * | | - | - | - | . | | | |

P<0.01 *; P<0.005**; P<0.001 ***; - Not considered factor.

3.2. Final biomass

Figure 11 shows the final biomass of the aerial parts of the plant for each genotype. In 2017, dry biomass was distributed almost equally in leaf and stem fractions, showing mean values of 38.3 and 40.4 g in leaf and stem respectively (Fig 12A). In 2018, the bunches weights supposed about 25% of total dry weight (Fig 12B). In this year, total plant dry

matter was higher than in 2017 (94.5 and 78.7 g, respectively), mainly because the presence of bunches induced a change in the dry matter distribution among the different aerial parts of the plants.

The total stem dry biomass showed a wide range, ranged between 20 and 45 g per plant (Fig 12). In 2017, genotypes showed higher stem biomass, highlighting the genotypes 232, 1048, and 326, which showed values around 45 g, in comparison to genotype RJ78 with a total stem weight of 30 g (Fig 12A). In the second year of study, genotype RJ51 had higher stem biomass (35 g) in contrast with genotypes RJ43 and 215 (21 g) (Fig 12B).

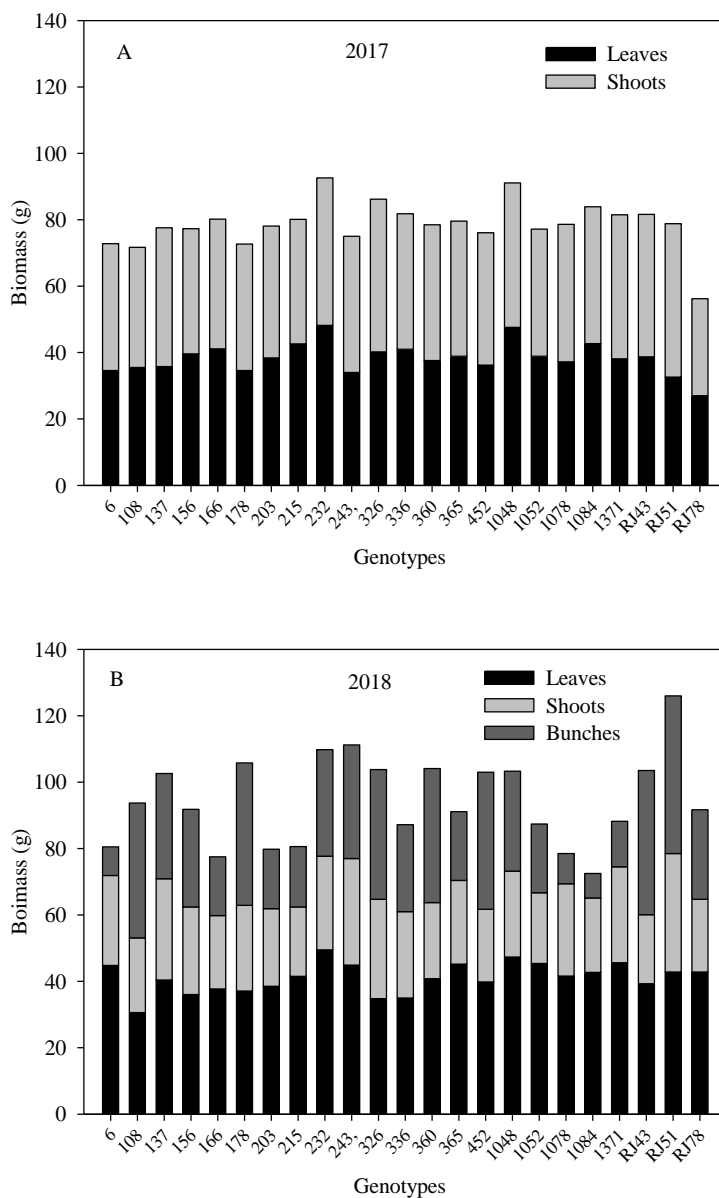


Figure 12. Total dry biomass divided in leaves (black bars), shoots (grey bars) and bunches (dark bars) measured at the end of experiments in 2017 (A) and 2018 (B) for each genotype (N=5).

The bunches weight presented large variations between genotypes (Fig 12B), ranged from 40 g in the genotypes RJ51, RJ43, and 178 to 10 g in the genotypes 6, 1078, and 1084. The total leaf dry weight varied between genotypes and years (Fig 12), and the two most productive genotypes were conserved during both years (232 and 1048, respectively). The total aerial dry biomass varied between 55 and 110 g.

During the season 2017, genotypes more productive in terms of total biomass were 232 and 1048 with a production upper than 90 g in contrast with genotype RJ78 (56 g). In the second season (2018), the genotype RJ51 was the most productive (126 g) in contrast with genotype 166 (78 g). In general, there was no clear relationship between years in terms of total biomass production. However, some high productivity genotypes (232 and 326) showed high biomass production both years. Two-way ANOVA (Year x Genotype) showed genotype and the year effect significant for the total dry biomass, and for stem and leaf weight. Bunches weight effect were not significant (Table 11) and the interaction between year and genotype effects were significant only for leaves weight (p-value <0.01).

3.3. Stomatal conductance, photosynthesis and WUE_i

The means of stomatal conductance, photosynthesis, and WUE_i are presented separated by years in the Fig 13. The water stress treatment reduced g_s by about 65% as mean (Fig 13A and 13B). This reduction in g_s was accompanied to a reduction of A_N , from 13.6 $\mu\text{mol m}^{-2}\text{s}^{-2}$ in well-watered (WW) to 7.8 in moderate water stress (MWS) (reduction of 57%). The higher impact of water stress in g_s compared to A_N implied an increment on WUE_i, which ranged between 55 to 87 $\mu\text{mol mol}^{-1}$.

The genotype effect was significant in g_s (p value <0.001), and in A_N (p-value <0.05) and was not significant in WUE_i (p-value <0.1). For both years, the maximal g_s in WW was around 0.32 $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$ and the minimal 0.21 $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$ (Fig 13A-B). These differences were also clear in A_N and WUE_i (Fig 13C-F). For example, under WW condition, genotype RJ78 showed a higher g_s and lower WUE_i both years, with 48.8 and 50.6 $\mu\text{mol CO}_2 \text{mol H}_2\text{O}^{-1}$, respectively. In contrast, genotype 1052 showed a low g_s both years (0.21 and 0.24 $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$) and not a high WUE_i (57 and 52 $\mu\text{mol CO}_2 \text{mol H}_2\text{O}^{-1}$, respectively). Under MWS treatment, the variation of WUE_i was around 20 $\mu\text{mol CO}_2 \text{mol H}_2\text{O}^{-1}$, reaching first year study genotypes 1052 and 156 around 90 $\mu\text{mol CO}_2 \text{mol H}_2\text{O}^{-1}$, and genotypes 1371 and 6 to 70 $\mu\text{mol CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$. The second year of study,

genotypes 1048 and 232 had the maximal WUE_i (around $100 \mu\text{mol CO}_2 \text{ mol H}_2\text{O}^{-1}$) compared to other genotypes 336 and 243 ($80 \mu\text{mol CO}_2 \text{ mol H}_2\text{O}^{-1}$). Against, the g_s level that arises each genotypes highly conditioned the resultant WUE_i . In this water status treatment, values between years were poorly conserved (Fig 13E-F).

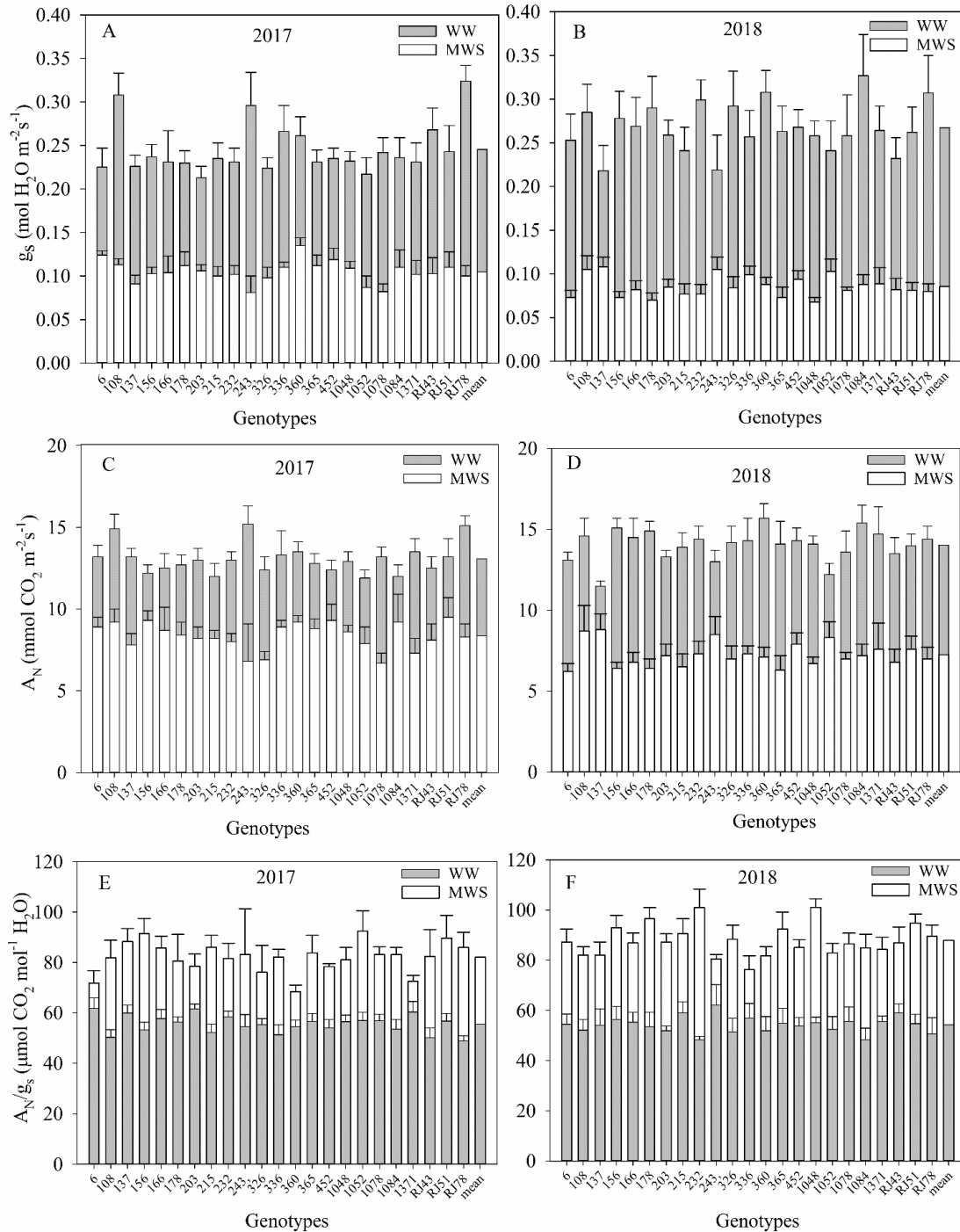


Figure 13. Leaf stomatal conductance (g_s), net photosynthesis rates (A_N) and intrinsic water use efficiency (WUE_i) for each genotype measured in 2017 (A,C,E) and 2018(B,D,F) in well-watered (WW) (grey bars) and moderate water stress (MWS) (white bars). Values are mean \pm standard deviation

A three-way ANOVA (Year x irrigation treatment x Genotype) showed that irrigation treatment and Genotype effects were statistically significant for A_N , g_s , and WUE_i . The Year effect was significant only for WUE_i (Table 12).

As reported before, the relationship between WUE_i and g_s was strongly significant. In consequence, to overcome the WUE_i variability induced by the range of variation of g_s during measurements, each genotype was characterised following its residuals of a general WUE_i - g_s relationship, in terms of percentage. This presents the advantage of characterize each genotype in respect to the general dependency of WUE_i over g_s .

The Fig.13 synthetises the differences in residuals between genotypes. In this way, it was feasible to distinguish four different types of behaviour: high and low WUE_i in both WW and MWS, and its combinations (WW+/MWS- and WW+/MWS-). Interestingly, there was no general relationship between the performance of genotypes in WW against MWS conditions but also some genotype shows better WUE_i under both conditions. The total variability found was about 15% in WW condition, with genotypes 243 and 1052 showing the most extreme values (-7 and +8%, respectively). In MWS condition, the total variability found was similar to WW condition, with genotype 156 as the most efficient (+6%) compared to genotype 360 (-8%) (Fig 14).

Using the WUE_i and g_s data generated during 2017 and 2018 for each genotype, we performed a particular lineal model to predict the resultant WUE_i as a function of g_s . Introducing the natural logarithm to this relationship allows the linearization of the relationship between the two variables. This model showed a wide variability for the particular genotype slopes, ranging from -3.2 for genotype 156 to -2.0 to genotype 243 (Fig 15).

The predicted WUE_i values for every genotype were obtained and ranked for a large range of g_s , from water stress to no water limitations (Table 13). Similar to general trend residuals, three different behaviours were observed: First, some genotypes performed always as highly efficient in the whole range of g_s , like genotypes 232 or RJ51. Secondly, others are always less efficient, like 326 or RJ43. Thirdly, some genotypes present a clear trade-off along the whole range of g_s : the highly efficient at low g_s and lowly efficient at high g_s (as 1048 genotype), or inversely, like 108.

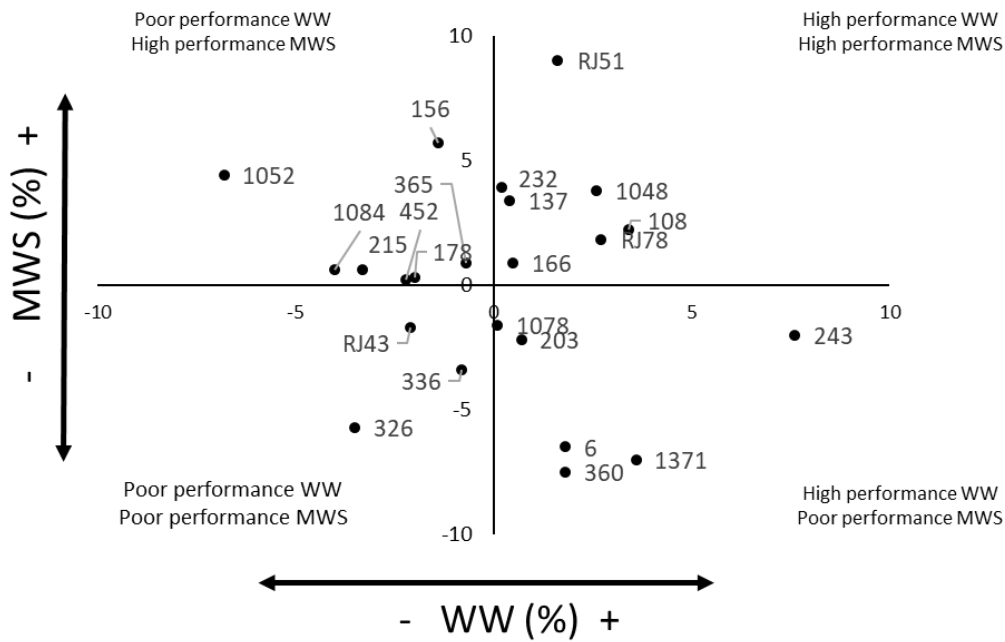


Figure 14. Residuals of the general WUE_i over g_s model expressed in terms of percentage for every genotype in WW (axis X) and MWS (axis Y) (See M&M section). Values are the mean ($n=10$) of the two experimental years.

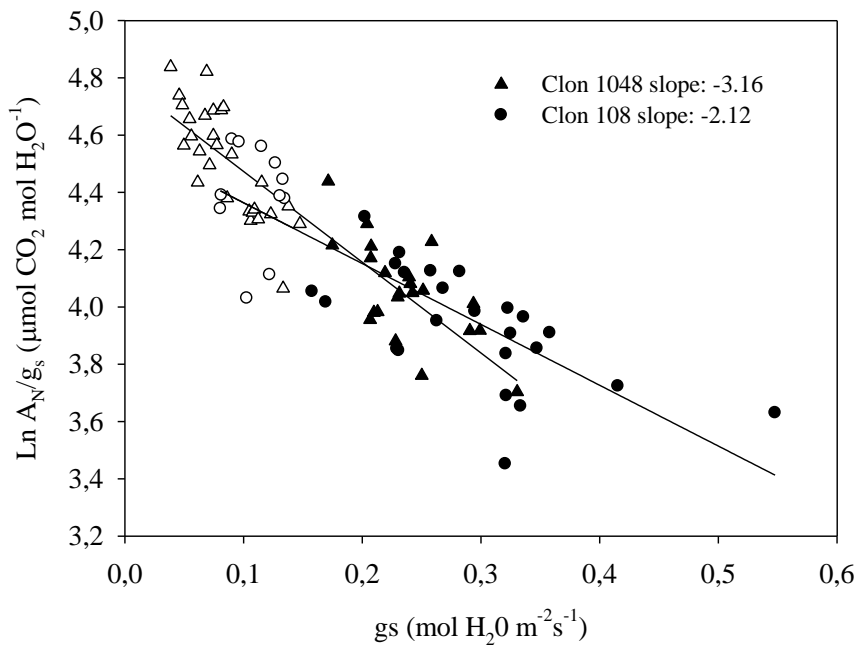


Figure 15. An example of the relationship linearized regressions between WUE_i and g_s using the natural logarithm of WUE_i of two contrasting clones. Filled symbols correspond to well-watered conditions; empty symbols correspond to moderate water stress conditions.

Table 13. The relative position of WUE_i predicted for each genotype linear regression at different g_s . The maximal, minimal, and the mean value of all genotypes were including in $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$.

| Genotype | g_s ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) | | | | | | |
|-------------|---|-------|-------|-------|-------|-------|-------|
| | 0.075 | 0.100 | 0.150 | 0.200 | 0.250 | 0.300 | 0.350 |
| 6 | 17 | 17 | 16 | 13 | 12 | 11 | 11 |
| 108 | 19 | 19 | 10 | 6 | 6 | 4 | 4 |
| 137 | 5 | 5 | 8 | 14 | 15 | 16 | 17 |
| 156 | 2 | 2 | 4 | 16 | 20 | 22 | 21 |
| 166 | 13 | 11 | 9 | 3 | 5 | 6 | 6 |
| 178 | 7 | 6 | 6 | 8 | 13 | 13 | 13 |
| 203 | 15 | 14 | 14 | 10 | 8 | 9 | 9 |
| 215 | 11 | 12 | 19 | 20 | 21 | 19 | 18 |
| 232 | 3 | 3 | 3 | 5 | 9 | 12 | 12 |
| 243 | 21 | 20 | 11 | 4 | 2 | 2 | 1 |
| 326 | 20 | 21 | 23 | 21 | 17 | 15 | 15 |
| 336 | 16 | 15 | 15 | 11 | 11 | 10 | 10 |
| 360 | 23 | 23 | 22 | 17 | 7 | 7 | 7 |
| 365 | 4 | 4 | 7 | 15 | 16 | 17 | 19 |
| 452 | 8 | 10 | 12 | 19 | 19 | 18 | 16 |
| 1048 | 1 | 1 | 2 | 9 | 17 | 21 | 22 |
| 1052 | 6 | 7 | 13 | 23 | 23 | 23 | 23 |
| 1078 | 18 | 18 | 17 | 12 | 10 | 8 | 8 |
| 1084 | 14 | 16 | 20 | 18 | 14 | 14 | 13 |
| 1371 | 22 | 22 | 18 | 7 | 4 | 3 | 3 |
| RJ43 | 12 | 13 | 21 | 22 | 22 | 20 | 20 |
| RJ51 | 9 | 8 | 1 | 1 | 1 | 1 | 2 |
| RJ78 | 10 | 9 | 5 | 2 | 3 | 5 | 5 |
| Max | 94.6 | 87.1 | 74.0 | 66.1 | 59.0 | 52.7 | 47.7 |
| Min | 80.7 | 76.6 | 68.8 | 60.3 | 51.3 | 43.7 | 37.2 |
| Mean | 86.8 | 81.3 | 71.3 | 62.6 | 54.9 | 48.3 | 42.4 |

4.5 Genotype variations in water use efficiency correspond with photosynthetic traits in Tempranillo grapevine clones

4.5.1 Plant water status

Midday stem water potential was assessed to ensure that the water treatments were established (Table 14). Average Ψ_{stem} across all six genotypes was -0.64 MPa during the well-watered (WW) period, and -1.29 MPa under moderate water stress (MWS; $p < 0.001$). We also observed the effect of the irrigation on the stomatal conductance (g_s), with g_s systematically over $0.2 \text{ mol H}_2\text{O m}^{-2}\text{s}^{-1}$ under WW and under $0.15 \text{ mol H}_2\text{O m}^{-2}\text{s}^{-1}$ under MWS (Fig. 16, Table 15). Under MWS, genotypes RJ51 and 1048 presented the lowest values ($0.07\text{-}0.08 \text{ mol H}_2\text{O m}^{-2}\text{s}^{-1}$) and genotype 326 the highest ($0.11 \text{ mol H}_2\text{O m}^{-2}\text{s}^{-1}$).

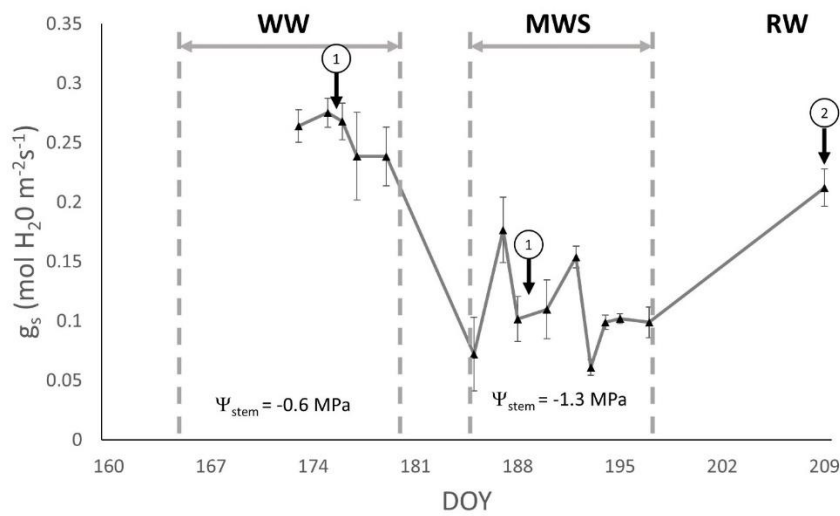


Figure 16. Variation of average stomatal conductance (g_s) along the measurement periods. The treatments were indicated by uppercase; WW: Well-watered period, MWS: Moderate water stress period, RW: Rewatering period. The measurements were done in the indicated days

Table 14. Stomatal conductance (g_s) and midday stem water potential (Ψ_{stem}) at two irrigation treatments.

| Genotype | g_s (mmol H ₂ O m ⁻² s ⁻¹) | | Ψ_{stem} (MPa) | |
|-------------|--|-----------------|---------------------|--------------|
| | WW | MWS | WW | MWS |
| 326 | 0.292 ± 0.040 | 0.111 ± 0.013a | -0.60 ± 0.21 | -1.39 ± 0.15 |
| 1048 | 0.258 ± 0.017 | 0.071 ± 0.005b | -0.74 ± 0.06 | -1.37 ± 0.24 |
| 1052 | 0.241 ± 0.034 | 0.116 ± 0.013ab | -0.59 ± 0.19 | -1.15 ± 0.20 |
| RJ43 | 0.232 ± 0.024 | 0.092 ± 0.009ab | -0.62 ± 0.23 | -1.12 ± 0.24 |
| RJ51 | 0.262 ± 0.029 | 0.081 ± 0.009b | -0.72 ± 0.15 | -1.36 ± 0.18 |
| RJ78 | 0.307 ± 0.043 | 0.101 ± 0.011ab | -0.61 ± 0.19 | -1.39 ± 0.15 |
| p-value | 0.50 | 0.05 | 0.64 | 0.12 |

4.5.2 Plant growth and final biomass

Plant growth parameters were calculated during each consecutive period of WW and MWS irrigation. Leaf expansion rate (LER) was almost twice as high during the WW period than the MWS period (29 cm² day⁻¹ vs. 15 cm² day⁻¹, respectively; $p > 0.001$). During the WW period, genotype 1052 exhibited the highest growth rate (37 cm² day⁻¹) and genotype 326 exhibited the lowest growth rate (24 cm² day⁻¹; Table 2). The variation in LER between genotypes was smaller during MWS; genotype RJ43 had the highest growth rate and genotype 326 had the lowest growth rate (19.5 and 11.5 cm² day⁻¹, respectively). The average reduction in LER due to the change in water regime from WW to MWS was around 50%, with the smallest reduction observed for genotype RJ43 (28%). Moreover, LMA ranged from between 77 to 86 g m⁻² under WW conditions. MWS increased the LMA by an average of 30%. Genotype 1052 exhibited the highest LMA during MWS and genotype RJ43, the lowest (128 and 105 g m⁻², respectively; Table 15).

Total aerial biomass was measured at the end of the season, considering the stem and leaf weight (aerial biomass) and grape production separately. The average dry weights for all genotypes were 73 g for the aerial biomass (leaf + stem) and 35 g for the grape bunches.

Genotypes RJ51 and 1048 produced the highest aerial biomass and genotype RJ43, the lowest (+15% and -10% of the average value, respectively; Fig. 17). The weight of the grape bunches varied highly between genotypes, with a difference of 235% between the most and least productive genotypes. Genotype RJ51 and genotype 1052 exhibited the highest and lowest total biomass production (+30% and -40% of the average value, respectively).

Table 15. Leaf expansion rate (LER) and leaf mass area (LMA) in each irrigation treatment.

| Genotype | Leaf expansion rate (LER) | | | LMA | | |
|-------------|--|---|----------------|----------------------------|-----------------------------|------------------|
| | WW (cm ² day ⁻¹) | MWS (cm ² day ⁻¹) | Reduction % | WW (g m ⁻²) | MWS (g m ⁻²) | Increment (%) |
| 326 | 24.0 ± 6.1 | 11.2 ± 3.6 | 53 ± 20 | 88.9 ± 3.6 | 100.1 ± 2.2 | 13 ± 4 |
| 1048 | 30.0 ± 6.2 | 17.3 ± 5.2 | 42 ± 11 | 80.2 ± 3.4 | 92.6 ± 5.9 | 16 ± 8 |
| 1052 | 36.6 ± 8.0 | 15.1 ± 1.8 | 59 ± 20 | 81.5 ± 4.0 | 104.2 ± 8.6 | 34 ± 14 |
| RJ43 | 27.1 ± 2.8 | 19.5 ± 3.7 | 28 ± 7 | 76.8 ± 2.2 | 85.6 ± 3.2 | 15 ± 5 |
| RJ51 | 31.7 ± 5.4 | 12.5 ± 3.2 | 60 ± 15 | 83.1 ± 5.7 | 90.6 ± 5.8 | 11 ± 9 |
| RJ78 | 26.2 ± 3.8 | 13.7 ± 2.4 | 48 ± 10 | 83.8 ± 5.2 | 92.1 ± 6.1 | 10 ± 5 |
| p-value | 0.66 | 0.6 | 0.42 | 0.35 | 0.46 | 0.28 |

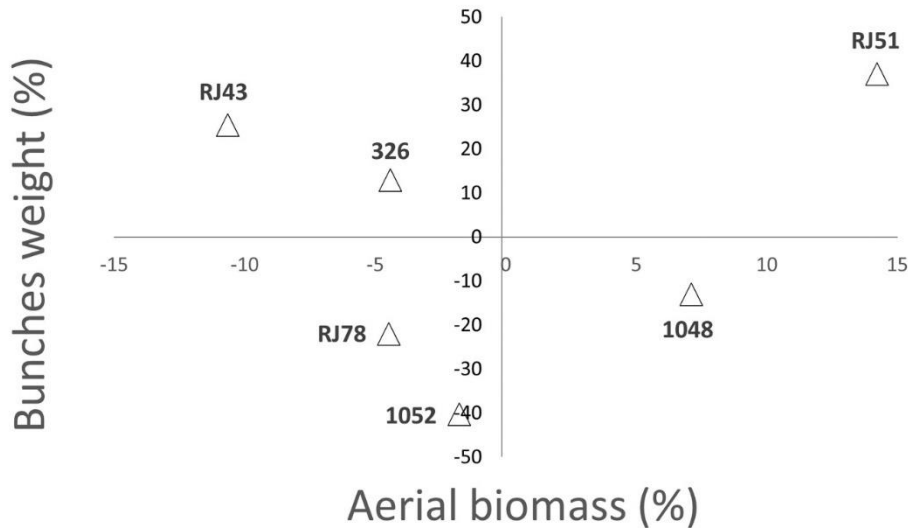


Figure 17. Representation of bunches and aerial biomass (leaves and stem) of the different genotypes, expressed as deviation in respect to the average of all them. Aerial biomass (axes X) and bunches weight (axes Y).

3.5.3 Hydraulic traits

Pressure-volume curves were performed for the rehydrated plants at the end of the experiment. The parameters derived from the P-V curves are shown in Table 16. Osmotic potential at full turgor (π_{FT}) ranged from -1.44 MPa in genotypes 1048 and 1052 to -1.14 MPa in genotype RJ78. Similar variability was observed for the turgor loss point Ψ_{TLP} , which ranged from -2.0 MPa for genotype 326 to -1.6 MPa for genotype RJ78. The elasticity modulus (e) also varied significantly between plants of the same genotype, with the genotype average varying from 15 MPa for genotypes 326, 1052 and RJ51 to 11 MPa for genotypes RJ43 and RJ78. Capacitance at full turgor (C_{FT}^*) was highest for genotype RJ78 ($0.63 \text{ mol m}^{-2} \text{ MPa}^{-1}$) and lowest for genotype 326 ($0.45 \text{ mol m}^{-2} \text{ MPa}^{-1}$). Capacitance at the turgor loss point ranged from $0.08 \sim 0.12 \text{ MPa}^{-1}$ across the six genotypes, with genotypes RJ51 and 1052 exhibiting the highest values. The symplasmic pathway (s_f) was estimated to represent about 30-40% of total leaf water for all six genotypes; genotypes 326 and RJ78 exhibited the lowest s_f values and genotypes 1048 and 1052, the highest (0.3 and 0.4, respectively).

Table 16. Average turgor weight divided by dry weight (TW/DW), osmotic potential at full turgor and at turgor loss point (Ψ_{FT} and Ψ_{TLP}), elasticity modulus (ϵ) and symplastic pathway (s_f) values for each genotype.

| Genotype | TW/DW | Π_{FT} (MPa) | Ψ_{TLP} (MPa) | ϵ (MPa) | s_f |
|----------|-------------|---------------------|-----------------------|---------------------|-------------|
| 326 | 2.78 ± 0.18 | -1.39 ± 0.13 | -2.04 ± 0.19 | 14.87 ± 1.22 | 0.31 ± 0.02 |
| 1048 | 2.89 ± 0.10 | -1.44 ± 0.09 | -1.99 ± 0.11 | 13.57 ± 2.41 | 0.41 ± 0.04 |
| 1052 | 2.85 ± 0.02 | -1.44 ± 0.18 | -1.94 ± 0.16 | 15.04 ± 4.48 | 0.41 ± 0.03 |
| RJ43 | 2.86 ± 0.05 | -1.19 ± 0.14 | -1.76 ± 0.11 | 10.93 ± 1.10 | 0.34 ± 0.05 |
| RJ51 | 2.71 ± 0.12 | -1.30 ± 0.16 | -1.76 ± 0.12 | 15.11 ± 3.02 | 0.37 ± 0.05 |
| RJ78 | 2.99 ± 0.09 | -1.14 ± 0.03 | -1.64 ± 0.01 | 11.91 ± 1.52 | 0.30 ± 0.02 |
| p-value | 0.51 | 0.45 | 0.20 | 0.70 | 0.22 |

4.5.4 Photosynthetic traits

The net photosynthetic response to variation in ambient CO₂ (A_N/C_i curves) was measured for all six genotypes at the end of the season, after rewatering. The parameters derived from the curve fitting method are presented in Table 17. The maximum photosynthetic rate (A_{max}) varied slightly between genotypes, ranging from 42 (1052) to 33.1 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (RJ43), though these differences were not statistically significant ($p > 0.05$). However, the maximum carboxylation velocity of Rubisco (V_{cmax}) varied significantly between genotypes ($p < 0.05$) and ranged from 115 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (genotype 326) to 72 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (genotype RJ51). The maximal electron transport rate (J) exhibited a similar range of variation ($p = 0.08$). Genotype RJ51 exhibited the highest mesophyll conductance (g_m) and genotypes RJ43, RJ78 and 1048, the lowest ($p = 0.01$). Leaf respiration (R) also varied significantly between genotypes ($p = 0.03$); genotype 1048 had the lowest R value (0.2 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and RJ78, the highest (0.6 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$).

Table 17. Average photosynthetic traits derived from A_N-C_i curves for each genotype; Maximal photosynthesis (A_{max}), maximal carboxylation velocity (V_{cmax}), maximal electron transport rate (J), mesophyll conductance (g_m) and leaf respiration (R)

| Genotype | A_{max} ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) | V_{cmax} ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) | J_{max} ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) | g_m ($\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) | R ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) |
|----------|---|--|---|--|---|
| 326 | 34.2 ± 3.0 | 114.5 ± 5.9a | 120.0 ± 10.4 | 0.180 ± 0.064ab | 1.00 ± 0.08bc |
| 1048 | 38.2 ± 4.2 | 76.7 ± 7.8b | 92.0 ± 11.1 | 0.127 ± 0.013b | 0.56 ± 0.04c |
| 1052 | 41.7 ± 2.2 | 80.1 ± 10.0ab | 90.9 ± 13.2 | 0.187 ± 0.051ab | 1.00 ± 0.07bc |
| RJ43 | 33.1 ± 3.8 | 79.6 ± 5.8b | 108.4 ± 8.9 | 0.095 ± 0.016b | 1.10 ± 0.05ab |
| RJ51 | 34.5 ± 4.6 | 72.4 ± 1.6b | 85.7 ± 1.3 | 0.332 ± 0.082a | 0.92 ± 0.06bc |
| RJ78 | 35.5 ± 4.2 | 101.2 ± 10.7ab | 111.1 ± 6.6 | 0.104 ± 0.011b | 1.36 ± 0.07a |
| p-value | 0.57 | 0.046 | 0.088 | 0.013 | 0.031 |

4.5.5 Water use efficiency

Genotypic variability in WUE was assessed at the leaf gas exchange level (WUE_i) and whole plant level (WUE_{WP} ; Table 18). Average WUE_i under WW conditions was around $55 \mu\text{mol CO}_2 \text{ mol H}_2\text{O}^{-1}$, and ranged from $51 \mu\text{mol CO}_2 \text{ mol H}_2\text{O}^{-1}$ for genotype RJ78 and genotype 326 to $59 \mu\text{mol CO}_2 \text{ mol H}_2\text{O}^{-1}$ for genotype RJ51. Higher variability was observed during the MWS period ($p < 0.005$), with a maximal value of $100 \mu\text{mol CO}_2 \text{ mol H}_2\text{O}^{-1}$ in genotype 1048 and lowest value of $80 \mu\text{mol CO}_2 \text{ mol H}_2\text{O}^{-1}$ in genotypes 326 and 1052. At the whole plant level, the average WUE_{WP} was $0.65 \text{ g dry weight L}^{-1}$. Genotypes RJ51 and RJ78 exhibited the highest and lowest WUE_{WP} ($0.79 \text{ g dry weight L}^{-1}$ and $0.57 \text{ g dry weight L}^{-1}$, respectively). WUE_{WP} presented high variability between the six genotypes; however, high plant-to-plant variability reduced the significance of the differences between genotypes.

Table 18. Leaf (WUE_i) and whole plant water use efficiency of each genotype.

| Genotype | WUE_i ($\mu\text{mol CO}_2 \text{ mol H}_2\text{O}^{-1}$) | | Whole Plant (g dry weight L^{-1}) |
|----------|--|-------------------------|--|
| | WW | MWS | |
| 326 | 51.4 ± 5.4 | $80.2 \pm 4.1\text{bc}$ | 0.65 ± 0.10 |
| 1048 | 55.0 ± 2.3 | $98.4 \pm 3.1\text{a}$ | 0.65 ± 0.07 |
| 1052 | 52.4 ± 5.2 | $80.5 \pm 4.7\text{c}$ | 0.55 ± 0.06 |
| RJ43 | 59.1 ± 3.6 | $81.7 \pm 3.8\text{bc}$ | 0.65 ± 0.08 |
| RJ51 | 54.7 ± 3.8 | $94.9 \pm 3.4\text{ab}$ | 0.79 ± 0.09 |
| RJ78 | 50.6 ± 6.5 | $84.3 \pm 4.9\text{bc}$ | 0.57 ± 0.10 |
| p-value | 0.56 | 0.003 | 0.44 |
| Range | 8 (15%) | 20 (23%) | 0.24 (37%) |

Chapter 5: General discussion

This general discussion is structured in five sections that cover from the variability in water use efficiency, to the sources of variation and their physiological bases.

The general theme of the thesis is a contribution to the set of challenges of viticulture in semi-arid areas, where water resources are already scarce and are threatened by the incidence of Climate Change (IPCC, 2021). The grapevine, due to its high economic impact and its wide distribution in these areas, is a highly vulnerable crop (Mozell et al., 2014).

Previous studies have evaluated the behavior of different grapevine varieties, exploring the interest of certain cultivars with greater tolerance to water stress (Bota et al., 2016; Haider et al. al., 2017; Shahmoradi et al., 2019; Dayer at., 2020; Flores-Sarasa et al., 2020). The main problem that limits the applicability of these studies is that the substitution of some varieties for others is not easy. For example, in Spain, the different protection figures (such as the different DOs or IGPs) limit the varieties that can be cultivated in each region. In addition, there are preferences of each specific market for certain varieties, since consumers tend to associate a specific variety with their expectations in the quality of a wine (Eibach & Töpfer, 2015). For this reason, although there are thousands of varieties suitable for viticulture, a few elite varieties take a large part of the global vineyard area. In Spain, four of these varieties (Airén, Tempranillo, Bobal and Garnacha) involve more than 60% of the total vineyard area (Ibañez et al., 2015). Therefore, it is necessary to explore intravarietal genetic variability in aspects related to tolerance to water stress or more water use efficient (WUE) based on measurements of photosynthesis and leaf transpiration. This approach tries to overcome the drawbacks of the realistic evaluation in the field, among which the difficulty of evaluating the real consumption of water and the variability in production induced by the variability of environmental factors throughout the crop stand out.

This thesis is focused on the genetic improvement of the grapevine, using the WUE as a selection criterion. The evaluation of the WUE can be done at different levels (MyM), but in this thesis the genotypes have been characterized by their intrinsic WUE (WUE_i , calculated as the ratio A_N/g_s , measured at the leaf level). In this way, the net photosynthesis (A_N) is used as an approximation to biomass gain while stomatal conductance (g_s) is used as an indicator of potential water losses due to transpiration. This measurement requires to measure the leaf gas exchange fluxes, which implies the use of fragile and complex equipment, but offering a high resolution at the scale of seconds,

allowing to expand the number of genotypes evaluated. For this reason, WUE_i has been widely used for the evaluation of different species (Gonzalez de Andres et al., 2018; Leakey et al., 2019), varieties of the same crop (Lanoue et al., 2017; Zhao et al., 2020), clones or genotypes of the same variety (Santos et al., 2021; Buesa et al., 2021) and rootstocks (Wang et al. al., 2018; Liu et al., 2019).

The main novelty of this work is that, for the first time, an intra-varietal selection has been carried out for this parameter, that is, an evaluation of different genotypes belonging to the same vine variety, in this case Tempranillo. For this, the different studies that make up each chapter have been carried out on multiple collections of clones established in the field in different institutions and locations, both public (the Institute of Wine and Viticulture Sciences) and private (Bodegas Roda and Viveros Provedo), as well as in experiments carried out under more controlled conditions in pots. The joint discussion of these studies is addressed below.

5.1.1 Intercultivar vs intracultivar variability of WUE

To know what extent intravarietal variability exists in WUE compared to multivarietal studies was one of the first objectives of this Thesis (chapter 1). For this, the results obtained in a collection of 23 commercial varieties were compared with as many clonal lines evaluated in situ, under field conditions and grafted on a similar rootstock. The results indicated that the dispersion of the data obtained, determined by its coefficient of variation on WUE with respect to the general average, was similar among varieties and clones of Tempranillo, reaching approximately an 80% inside the collection of Tempranillo clones compared with the collection of varieties. In addition, in a similar water stress range, WUE in the clone collection ranged between 80 and 136 $\mu\text{mol mol}^{-1}$, while in the multivarietal collection this range was extended between 69 and 118 $\mu\text{mol mol}^{-1}$ (Tortosa et al., 2016).

On one hand, considering the results, it is surprising that the intra-varietal genetic variability of the WUE_i is almost as wide as the inter-varietal one, so exploring this source of variation in a selection program can be promising. This wide variation was also revealed in previous selections programs attending to the selection by production or grape quality parameters, and may be related to the age of this variety used in viticulture and its wide geographic distribution (Carbonell-Bejerano et al., 2016). On the other hand, these data are also an exponent of the interesting wealth of genetic variability that accumulates

over time in a genotype (a clone) that reproduces asexually and that is worth exploring using the current most advanced genomics tools. In the specific case of the Tempranillo variety, it must be considered that the collections of clones studied do not cover the total set of clones registered in this variety from Australia to California or Spain.

This first result of this thesis represents a significant advance in the general interest of exploring the "intra-varietal" variations in other cultivated varieties and reproduced habitually as clones of greater or lesser reputation. Our team has recently been able to verify those variations in other varieties such as Garnacha (Buesa et al., 2021) and Monastrell (Buesa et al., 2022, in press), so, this variability is therefore of the greatest interest.

5.2. Intracultivar Variability of WUE inside Tempranillo cv.

In this thesis, the WUE assessment is made as the ratio between photosynthesis and stomatal conductance for different reasons already discussed (Chapter 4.1). In this sense, we have to consider that the main determinant of the WUE_i is g_s , as can be deduced from its wide variation and the strong correlation between both parameters (Fig. 18, $p < 0001$). In addition, as mentioned above, the specific value of the WUE is strongly determined by the g_s , so for the evaluation of genotypes by WUE it is essential to consider g_s which reflects the water status of the plant.

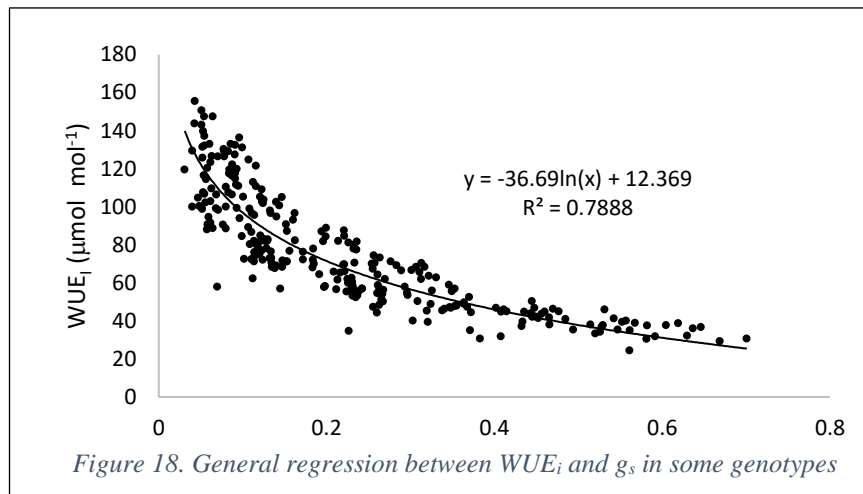


Figure 18. General regression between WUE_i and g_s in some genotypes

The study of the relationship between these two variables, widely described (see Medrano et al, 2002), establishes, for practical purposes, three differentiated water stress ranges; severe water stress when g_s is less than $0.075 \text{ mol m}^{-2}\text{s}^{-1}$, moderate water stress, with g_s between 0.075 and $0.150 \text{ mol m}^{-2}\text{s}^{-1}$, and plants with little or no water stress, when

g_s is greater than $0.150 \text{ mol m}^{-2}\text{s}^{-1}$ (Medrano et al., 2002; Flexas et al., 2002; Cifre et al., 2004). Therefore, for a comparison of the WUE of the different genotypes, it is necessary to consider the range of g_s at the time of the evaluation. As a novelty, in this Thesis we proposed a novel method to avoid the small trends that continue to exist if we simply compare among plants with water stress and without such stress. The method followed in this Thesis consists of obtaining the relationship between WUE_i and g_s in each data set, usually showing regression coefficients above 0.8. This joint relationship allows us to identify the adjustment curve that allows us to predict the expected value of WUE_i for a given value of g_s . From this relationship and the specific g_s of a specific clone we can calculate the expected WUE_i , provided by the general model. Comparing the measured WUE_i with respect to the expected WUE_i , the deviation (positive or negative) can be calculated and expressed as a percentage (positive or negative) that characterizes the behavior of a certain clone with respect to the average of the set, thus being able to establish an order of genotypes in every situation. This percentage facilitates the quantification of the differentiation margin in the WUE for each of the clones. This pathway allows clone behaviors to be quantified, enabling to calculate differences of up to 20% among the most efficient genotypes compared to those that are least efficient, as shown in Chapter 4.1.

In the present thesis, more than 120 genotypes have been measured in the field, including in plots of La Grajera (Logroño, La Rioja, Spain), Viveros Provedo (Varea, La Rioja, Spain), Bodegas Roda (Haro, La Rioja, Spain) and Vitis Navarra (Larraga, Navarra, Spain), of which a core selection was published during the completion of this thesis (Tortosa et al., 2019a; 2019b; 2020). From this collection, only a selection of genotypes has been evaluated during time series of 3 to 5 years to contrast its stability over time, as mentioned above.

The comparative study in different years allows to measure the stability of the assessments of each clone. These time series showed how some genotypes such as RJ51, 463 and 44 have sustained WUE_i above the average (+13 to +8%) while genotypes such as 260 or 215 remained consistently below the adjusted general average (-6 to -9%). However, considering the differences among genotypes throughout the different campaigns (see tables 5 and 6, chapter 4.2), different behaviors of the same genotype in different water status are observed. For example, the 365 genotype presents a positive percentage (+7%) in conditions of high-water availability and its behavior falls (-4%)

under water stress, contrary to what happens with other genotypes (see 108 as an example).

The high number of measurements accumulated throughout the different campaigns, together with the variations registered in the water status of the plots, it is possible to analyze the behavior of the WUE_i of certain genotypes in a wide range of water availability conditions. This genotype-by-genotype analysis for the values obtained from the WUE_i under different conditions was also carried out in a regression contrast method, comparing the slopes of the linear fit between both, WUE_i and g_s , and the independent terms between them (see chapter 4.3), finding significant differences between the curves of regression of the different genotypes. Subsequently, based on the measured data, it was decided to model the response of the WUE_i to different levels of g_s representative of three water status, corresponding to severe, moderate or low water limitation water stress. The model makes possible to analyze the behavior of the different genotypes (see chapters 4.3 and 4.4), and clearly identify genotypes with higher WUE_i (more adapted to water stress) and others whose efficiency improves when water availability is greater, as in the case of the RJ78 genotypes versus the 1052 genotype (Chapter 4.3).

In parallel, a collection of genotypes was selected the first years and planted in pots in subsequent years, although, as has been described, pots conditions imply an alteration of the natural conditions with respect to the field that affects different aspects of its physiology (Poorter et al., 2012). However, pot experiments, under controlled conditions, allows clones to be compared in a more homogeneous environment, equalizing fertilization and controlling the water status imposed at each moment (chapter 4.4). Even under these conditions, the repeated evaluation along years again produced significant variations due to “year effect”, both in biomass production and in WUE_i . Despite these differences between seasons, WUE_i variability was observed between genotypes under conditions of high-water availability (243, +8% vs. 1084, -5%) and under water stress conditions (RJ51, +10% vs. 6, - 5%). Analyzing the averages, the differences in behavior in field and pot conditions are evident (Table 19), although genotypes such as 326 or 1084 show a consistent response between pots and field conditions under no water stress. Under moderate water stress conditions, the 1048 genotype also shows higher WUE in pots and field environments.

Table 19. Comparison of genotype WUE behavior considering plant water status and growth environments (field and pots).

| Genotypes | Field | | Pot | |
|-----------|-------------|------------|------------|------------|
| | WW | MWS | WW | MWS |
| 6 | 9.7 ± 2.0 | 3.9 ± 2.8 | 1.2 ± 2.5 | -4.7 ± 3.6 |
| 108 | -1.1 ± 2.8 | 6.0 ± 3.8 | 3.1 ± 3.5 | 4.4 ± 5.8 |
| 137 | -23.1 ± 0.0 | | 0.1 ± 3.0 | 5.3 ± 3.9 |
| 156 | -5.7 ± 0.0 | | -4.4 ± 3.1 | 5.8 ± 3.4 |
| 166 | -3.2 ± 0.0 | | -0.3 ± 2.5 | 0.6 ± 2.6 |
| 178 | 7.3 ± 0.0 | | -2.7 ± 2.4 | 2.0 ± 3.5 |
| 203 | 5.0 ± 0.0 | | 0.0 ± 2.1 | -0.5 ± 3.5 |
| 215 | -6.9 ± 0.0 | | -5.0 ± 3.2 | 2.2 ± 2.9 |
| 232 | | 4.3 ± 3.2 | 0.2 ± 2.3 | 6.2 ± 3.5 |
| 243 | -5.8 ± 0.0 | | 7.7 ± 4.8 | -0.3 ± 9.0 |
| 326 | -15.9 ± 0.0 | | -4.5 ± 2.8 | -3.6 ± 4.7 |
| 336 | 0.0 ± 3.6 | -0.8 ± 3.8 | -1.8 ± 5.5 | 0.9 ± 2.7 |
| 360 | -12.5 ± 0.0 | | 1.0 ± 2.2 | -5.2 ± 2.6 |
| 365 | 6.8 ± 0.9 | -3.5 ± 1.1 | -1.8 ± 3.0 | 2.7 ± 3.6 |
| 452 | -0.1 ± 0.0 | | -3.3 ± 2.8 | 1.8 ± 2.4 |
| 1048 | -4.5 ± 3.4 | 2.4 ± 3.4 | 1.1 ± 2.8 | 6.0 ± 2.5 |
| 1052 | 1.2 ± 4.6 | 6.4 ± 1.9 | -7.7 ± 1.7 | 5.0 ± 4.1 |
| 1078 | 11.1 ± 2.9 | 1.5 ± 2.9 | 0.1 ± 2.9 | 0.6 ± 3.1 |
| 1084 | -2.4 ± 3.0 | 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 ± 4.0 |
| RJ78 | 0.5 ± 3.9 | -4.3 ± 5.3 | 3.7 ± 2.7 | 2.9 ± 3.6 |

Finally, the pot experiments allowed establishing degrees of gradual water stress that considerably increased the resolution of the WUE_i model with respect to g_s applied to each genotype (see chapter 6). This evaluation method, including values from different experiments, gains in robustness and allows us to conclude that genotypes such as RJ51 have a high efficiency when g_s is greater than $0.150 \text{ mol m}^{-2}\text{s}^{-1}$ and 1048 when g_s is less than $0.150 \text{ mol m}^{-2}\text{s}^{-1}$. There is no precedent for using models like these to compare the response of genotypes in WUE_i .

5.3.Sources of variation of WUE

It is widely known that factors such as environmental PAR radiation, measurement time, leaf age, fertilization and soil structure, and especially water status can alter the

specific value of the WUE_i and therefore the intraclonal evaluation of the WUE. (Farquhar et al., 2001; Kaiser et al., 2015;)

All this environmental variation has been revealed in numerous previous studies, where the high significance and magnitude of the "year" effect are cited, indicating that cumulative environmental factors over time and specific to each year, change the behavior of the evaluated genotypes. Thus, comparing different vine varieties, Soar et al. (2003); Tomas et al. (2014a); Bota et al. (2016); Romero et al. 2018; Migicovsky et al. (2021) show clear examples of the importance of this effect in the specific values of the WUE_i of different genotypes.

Chapter 4.3 compares a set of data corresponding to 10 clones of Tempranillo measured for 3 or 5 years, depending on the plot where they were located. The data show that the "year" effect has an important influence on the g_s , A_N and WUE_i measured for the different genotypes, in addition to an apparent effect on the variability observed in the plot (understood as sample deviation, coefficient of variation of the data or as a range between maximum and minimum). These results also show the difficulties of carrying out any selection program based on the application of phenotypic criteria in one or a few years, and highlight the need to carry out evaluations considering long time series in which conflicting results may occasionally be obtained (Medrano et al., 2003; Zhou et al., 2018).

However, in this same chapter, the variability observed in the WUE measured in leaves was compared with the WUE of the whole plant water use efficiency estimated by the ratio between harvest yield and water applied. Considering annual series of both parameters allowed a comparison of the associated environmental variability according to the selection criteria applied, based on the stability of the genotypes between years of the two parameters. Despite the high influence of the year effect in the two WUE, the data presented show how the yield has percentage variations 3 or 4 times higher in magnitude with respect to the WUE_i measured in leaves, which seems to indicate that the margins of error or environmental variability applied to the WUE_i are comparable even rather lower than expected when a selection is made by classical agronomic parameters.

Although establishing percentages above or below the general trend is a way to reduce environmental variability due to water status, it must be considered that there are many other factors that are a source of additional variability. For example, in Figure 19 data

referring to two different plots and differentiated by year of measurements are presented together. Note that the genotypes located in Roda (Roda) have values clearly below those located in La Grajera, (LG) even with similar stomatal conductances and the differences are accentuated for values of moderate or severe drought. It is not possible to conclude that these differences are due to the differentiated behavior of the genotypes present in one or another plot, but rather to environmental factors specific to each environment (soil, fertility, accumulated thermal integral, plant adaptation).

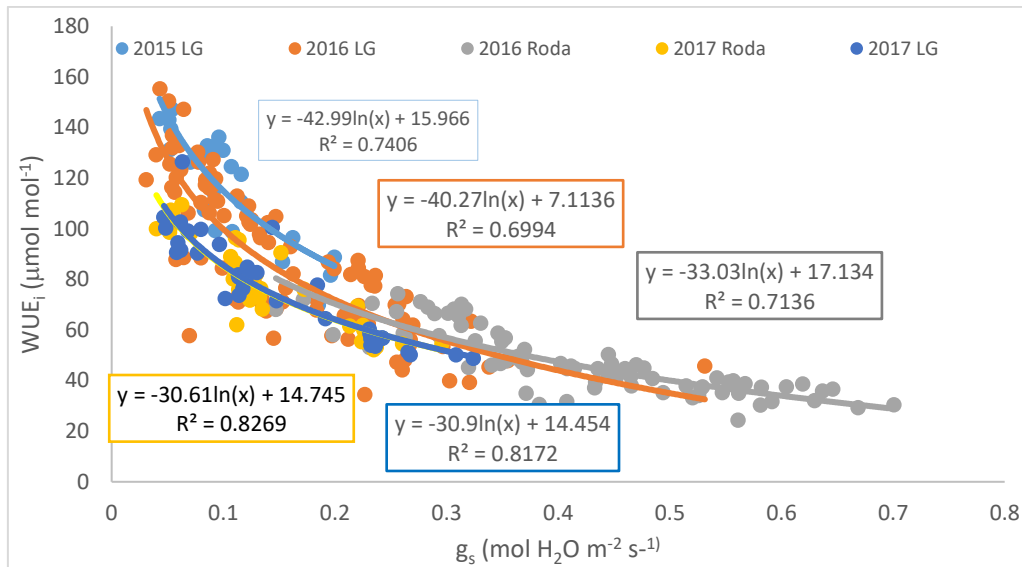


Figure 19. Relationship between WUE_i and g_s differentiating experimental sites and years. LG: La Grajera (Logroño, Rioja, Spain) Roda: Bodegas Roda (Haro, Rioja, Spain).

In conclusion, the evaluation of the genotypes in situ, that is, evaluating the behavior in the field, implies the interference of a considerable environmental variation and, above all, little controllable. Due to the importance of these variations, the comparison method, based on deviation percentages with respect to the general regression line, is a very useful and adequate evaluation method for the selection of genotypes.

5.4. Physiological traits explaining WUE observed variability

The identification of genotypes whose WUE_i is always consistently in the upper or lower part of the regression WUE_i vs g_s , allows us to select contrasted genotypes in order to study the physiological bases that determine these differences, which could be of great interest to improve the efficiency of the selection processes. In this sense, these genotypes were identified and potted experiments were carried out, focused on the study of physiological parameters related to WUE_i such as leaf hydraulics and photosynthetic characteristics (chapter 4.5).

Regarding the water relations characteristics, the first fact that should be noted is that the high plant-to-plant variability in parameters derived from P/V curves such as the osmotic potential at full turgor (Ψ_{OFT}), the point of loss of turgor (Ψ_{TLP}) and the modulus of elasticity (ϵ), which caused that the differences observed were not statistically significant between genotypes despite the wide ranges of variation observed. For example, the Ψ_{OFT} that has been related in the literature to stomatal closure in severe water stress (Martorell et al., 2015a; Esperón-Rodríguez et al., 2018). In our experiments it is possible to differentiate two groups of genotypes; the RJ43 and RJ78 with high Ψ_{OFT} (-1.15 MPa) compared to genotypes such as 326, 1048 and 1052 (-1.4 MPa). The ϵ , which has often been related to the thickness of the cell wall (Roig-Oliver et al., 2020; Nadal et al., 2018), also showed important variations, between 15 (RJ51 and 326) and 11 MPa (RJ43 and RJ78). However, none of the parameters studied was statistically significant. In this sense, the wide plant-plant variation induced by the environment does not allow establishing consistent relationships between the water parameters usually considered and the WUE_i in this group of genotypes.

The photosynthetic parameters were analyzed from the study of the response of the rate of photosynthesis to variable concentrations of CO_2 (curve of CO_2). The parameters deduced from A_N/C_i curves (V_{cmax} , g_m and R), showed, however, clear differences between genotypes. Although the differences in J_{max} were not statistically significant, a high positive correlation was obtained with respect to V_{cmax} in all the genotypes evaluated. The genotypes with high WUE_i (RJ51 and 1048) showed a low V_{cmax} and J_{max} , contrary to the genotype 326. This result could be related to the fact that the genotypes with a high V_{cmax} present, consequently, a reduction in C_i , which it usually implies a higher g_s , consequently reducing the value of the WUE_i (Flexas et al., 2010; Galmes et al., 2017).

Regarding the respiration rate, a relationship between the respiration rate and higher survival rates in the face of abiotic or biotic stresses, such as salinity or the presence of pathogens, has often been observed in previous literature (Atkin et al., 2015). However, under controlled conditions without other limitations beyond water availability, it seems reasonable to think that a decrease in leaf respiration increases the net carbon gain with the consequent implication in the improvement in biomass gain and therefore in the WUE , both in leaf and whole plant. Although the previous bibliography is still scarce, some authors have shown that leaf respiration can account for 10% of the total carbon balance of the vine plant (Hernández-Montes, 2017). In addition, in previous

studies, a lower respiration ratio per gram of leaf biomass corresponded to a higher WUE of the Garnacha variety compared to the Tempranillo variety (Hernandez-Montes et al., 2019). In other crops, for example in cereals (*Triticum* sp), the importance of respiration in the carbon balance and in the results in the WUE has been demonstrated using the carbon isotope discrimination technique (Aranjuelo et al., 2009). In our results, (Chapter 4.5), the high respiration rate corresponded in certain cases with a low accumulation of biomass and WUE, and on the contrary (low respiration and high WUE), indicating the important role of this parameter in the WUE of the plant.

Mesophyll conductance (g_m) defined the leaf capacity of CO_2 diffusion from the substomatal cavity to carboxylation sites in the stroma, including the intercellular spaces conductance, cell walls, and intracellular fluid (Flexas et al., 2008). Numerous studies point to interspecies and intraspecific diversity in g_m values (Flexas et al., 2009; Niinemets et al., 2009; Tomàs et al., 2014b; Han et al., 2018). These differences, and the g_m/g_s ratio has been related to a higher WUE, because greater internal diffusion allows greater C fixation with less stomatal opening (Medrano et al., 2002; Flexas et al., 2003; Flexas et al., 2010). The results presented in this thesis are clear, confirming that the higher g_m of the RJ51 genotype is related to its high WUE_i under the conditions maintained in the experiment.

The two genotypes selected for high WUE in the evaluation carried out in pots showed different physiological mechanisms according to the results. On one hand, the 1048 genotype showed a significant decrease in the respiratory rate measured in the leaf, which implies an improvement in the carbon balance and therefore in the WUE. On the other hand, while the RJ51 genotype was the one that showed a higher g_m , which implies a higher rate of photosynthesis for a similar g_s under these conditions and a high WUE. On the contrary, genotype 1052 showed the lowest g_m and the lowest WUE.

In conclusion, in the nuclear list of genotypes evaluated, differentiated physiological mechanisms have been observed for the optimization of the WUE, which involve controlling the losses of C or increasing the internal diffusion of CO_2 . These advances in the identification of the physiological basis of WUE_i are of great interest from a general physiology perspective, although they do not currently lead to an acceleration of the selection processes by WUE_i , since the determination of these parameters requires much more time and specialization than the determination direct from the WUE_i measured in the leaf. In any case, these results represent an advance in the knowledge of

the physiological bases of WUE_i , which also shows a clear variation between genotypes and that allows a better explanation of the differences observed between them.

5.5.Future challenges

In this thesis, the existence of intra varietal variability of WUE in *Vitis vinifera*, Tempranillo variety, was evidenced. Likewise, a very broad environmental influence of the WUE_i is verified, which limits the scope of the selection processes and forces determinations in different years and environmental conditions. A consistent relationship between certain parameters such as g_m and leaf respiration rate with a higher WUE_i is also demonstrated. In future works it would be necessary to go deep into the study of the genetic and epigenetic bases that justify these observed differences.

At the genetic level, it has been demonstrated for other authors how water stress causes variations in the expression of a large number of genes. In fact, information on this subject is increasing in publications that relate this gene expression to metabolic or physiological pathways (Deluc et al., 2009; Corso et al., 2015; Rocheta et al., 2016; Sharma et al., 2020). Likewise, differences in genetic expression have been demonstrated in the face of water stress related to stomatal control, WUE and other parameters (Dal Santo et al., 2016; Rocheta et al., 2016; Gambetta et al., 2020), and recently, Catacchio et al. (2019), have identified specific genes that explained the different response to water stress in two table grape varieties. These advances reinforce the interest of approaching with these new tools, the identification of the genes that justify the differences in the WUE demonstrated in the present thesis in the selected genotypes.

Another fundamental aspect of this thesis has been the usefulness of analyzing the results by contrasting the regression of the WUE_i against the g_s , as an indicator of the available water, given that this parameter is the most influential on the resulting WUE_i . This contrast in regression is also justified by the high values of the adjustment of the corresponding curve and allows approaching the analysis of the differences between genotypes in conditions of identical g_s , although similar values are not available in the field data. This approach is currently being applied in other selection programs. In this way, the study developed here is being extended to other major vine varieties such as Garnacha and Monstrell (Buesa et al., 2021) and to the effect of the different rootstocks in the USA (Buesa et al., 2022, In press).

Finally, the results obtained allow recommending a study of the physiological mechanisms that justify the WUE_i values of the differentiated genotypes, in order to broaden the search for characters or markers that are easy to measure massively and that allow expanding the number of evaluated genotypes in selection program based on WUE character.

Chapter 6: Conclusions

The preceding results and discussion allow us to conclude that intra cultivar selection is a potential tool to adapt the vineyard to water scarcity.

The main objective of this work was to check if variability exists in the WUE in a single grapevine cultivar, avoiding environmental effects in the evaluation of genotypes.

Considering all the work presented, the main conclusions drawn for each objective are as follow:

Objective 1. To study the genetic variability within Tempranillo cultivar in relation to a collection of multiple grapevine cultivars at field conditions.

1.1 Under similar environmental conditions and similar water stress, the WUE_i coefficient of variation between varieties shows values of 32%, while between genotypes of Tempranillo it is 26%, showing that there is enough variability to address a clonal selection program based on the WUE_i .

1.2 The variability of WUE in the varietal collection seems to be higher than that observed in the Tempranillo genotypes collection.

Objective 2. To establish a new method to evaluate genotype water use efficiency performance.

2.1 The high correlation observed between WUE_i against g_s allows to calculate the residuals as an effective tool to ranking genotypes at field conditions thus reducing the WUE water availability dependency.

2.2 When genotypes are measured over a wide range of water availability, individual models for each single genotype can be applied for the prediction and the quantification of genotypes performance for all water status.

Objective 3. To analyze the interaction environment vs. genotype on the WUE variability.

3.1 The sensitivity of g_s to water availability has an important effect on the WUE_i , probably underestimating the genotype effect and altering the resulting ranking.

3.2 An important year effect was also detected affecting net photosynthesis of all genotypes at the same g_s , probably due to accumulated thermal integral or plant adaptation during sprout.

3.3 The relationship between WUE_i and g_s always shows a good correlation when genotypes were evaluated at same conditions, year and experimental field, although not conserved between experimental fields and years.

3.4 Among years and for each genotype, variations in leaf WUE were lower than WUE_{crop} , suggesting that evaluations at leaf level could be a more accurate tool than agronomic traits for genotype breeding.

Objective 4. To compare the genotypes performance under field and pot conditions.

4.1 At field conditions, some genotypes, such as RJ51, 463 and 44, stand out for their high WUE_i , while other genotypes, such as 260 or 215, remained consistently below the adjusted general average.

4.2 At pot conditions, individual models of WUE_i showed that genotypes, such as RJ51, have a high WUE_i in well-watered conditions, while genotypes as 1048 presented high performance in moderate water conditions.

4.3 In general, the genotypes WUE_i behavior did not show a clear correlation between pot and field conditions, with some exceptions. These results suggested that precaution must be taken when performing breeding programmes at pot conditions.

Objective 5. To understand the physiological basis of the variations of water use efficiency in elite genotypes of Tempranillo.

5.1 At pot conditions, 1048 and RJ51 genotypes showed different physiological mechanisms that could explain this outstanding WUE_i , as respiration or mesophyll conductance to CO_2 .

References

- Acevedo-Opazo, C., Ortega-Farias, S., & Fuentes, S. (2010). Effects of grapevine (*Vitis vinifera* L.) water status on water consumption, vegetative growth and grape quality: An irrigation scheduling application to achieve regulated deficit irrigation. *Agricultural Water Management*, 97(7), 956-964.
- Albuquerque, C., Scoffoni, C., Brodersen, C. R., Buckley, T. N., Sack, L., & McElrone, A. J. (2020). Coordinated decline of leaf hydraulic and stomatal conductances under drought is not linked to leaf xylem embolism for different grapevine cultivars. *Journal of Experimental Botany*, 71(22), 7286-7300.
- Alsina, M.M., Smart, D.R., Bauerle, T., De Herralde, F., Biel, C., Stockert, C., Save, R. (2011). Seasonal changes of whole root system conductance by a drought-tolerant grape root system. *Journal of Experimental Botany*, 62:99–109.
- Anderson, K., & Aryal, N. R. (2013). Which winegrape varieties are grown where? A global empirical picture (p. 700). University of Adelaide Press.
- Aranjuelo, I., Cabrera-Bosquet, L., Mottaleb, S. A., Araus, J. L., & Nogues, S. (2009). $^{13}\text{C}/^{12}\text{C}$ isotope labeling to study carbon partitioning and dark respiration in cereals subjected to water stress. *Rapid Communications in Mass Spectrometry: An International Journal Devoted to the Rapid Dissemination of Up-to-the-Minute Research in Mass Spectrometry*, 23(17), 2819-2828.
- Arrizabalaga-Arriazu, M., Gomès, E., Morales, F., Irigoyen, J. J., Pascual, I., & Hilbert, G. (2020). High temperature and elevated carbon dioxide modify berry composition of different clones of grapevine (*Vitis vinifera* L.) cv. tempranillo. *Frontiers in plant science*, 11, 1888.
- Atkin, O. K., Bloomfield, K. J., Reich, P. B., Tjoelker, M. G., Asner, G. P., Bonal, D. & Zaragoza-Castells, J. (2015). Global variability in leaf respiration in relation to climate, plant functional types and leaf traits. *New Phytologist*, 206(2), 614-636.
- Autovino, D., Provenzano, G., Monserrat, J., Cots, L., & Barragán, J. (2016). Determining optimal seasonal irrigation depth based on field irrigation uniformity and economic evaluations: Application for onion crop. *Journal of Irrigation and Drainage Engineering*, 142(10), 04016037.

- Bacelar, E. A., Moutinho-Pereira, J. M., Gonçalves, B. C., Ferreira, H. F., & Correia, C. M. (2007). Changes in growth, gas exchange, xylem hydraulic properties and water use efficiency of three olive cultivars under contrasting water availability regimes. *Environmental and Experimental Botany*, 60(2), 183-192.
- Batchelor, C., Hoogeveen, J., Faurès, J. M., & Peiser, L. (2016). *Water accounting & auditing guidelines: A sourcebook (Vol. 43)*. Food & Agriculture Org.
- Bchir, A., Escalona, J. M., Gallé, A., Hernández-Montes, E., Tortosa, I., Braham, M., & Medrano, H. (2016). Carbon isotope discrimination ($\delta^{13}\text{C}$) as an indicator of vine water status and water use efficiency (WUE): Looking for the most representative sample and sampling time. *Agricultural water management*, 167, 11-20.
- Bellasio, C., Beerling, D. J., & Griffiths, H. (2016). An Excel tool for deriving key photosynthetic parameters from combined gas exchange and chlorophyll fluorescence: theory and practice. *Plant, cell & environment*, 39(6), 1180-1197.
- Bellvert, J., Mata, M., Vallverdú, X. et al. Optimizing precision irrigation of a vineyard to improve water use efficiency and profitability by using a decision-oriented vine water consumption model. *Precision Agriculture* 22, 319–341 (2021).
- Bota, J., Flexas, J., & Medrano, H. (2001). Genetic variability of photosynthesis and water use in Balearic grapevine cultivars. *Annals of Applied Biology*, 138(3), 353-361.
- Bota, J., Tomás, M., Flexas, J., Medrano, H., & Escalona, J. M. (2016). Differences among grapevine cultivars in their stomatal behavior and water use efficiency under progressive water stress. *Agricultural Water Management*, 164, 91-99.
- Bravdo, B., Lavee, S., & Samish, R. M. (2017). Analysis of water consumption of various grapevine cultivars. *VITIS-Journal of Grapevine Research*, 10(4), 279.
- Buesa, I., Mirás-Avalos, J. M., & Intrigliolo, D. S. (2020a). Row orientation effects on potted-vines performance and water-use efficiency. *Agricultural and Forest Meteorology*, 294, 108148.

- Buesa, I., Ballester, C., Mirás-Avalos, J. M., & Intrigliolo, D. S. (2020b). Effects of leaning grapevine canopy to the West on water use efficiency and yield under Mediterranean conditions. *Agricultural and Forest Meteorology*, 295, 108166.
- Buesa, I., Escalona, J. M., Tortosa, I., Marín, D., Loidi, M., Santesteban, L. G., Douthe, C. & Medrano, H. (2021). Intracultivar genetic diversity in grapevine: Water use efficiency variability within cv. Grenache. *Physiologia Plantarum*, 173(4), 2226-2237.
- Casassa, L. F., Keller, M., & Harbertson, J. F. (2015). Regulated deficit irrigation alters anthocyanins, tannins and sensory properties of Cabernet Sauvignon grapes and wines. *Molecules*, 20(5), 7820-7844.
- Catacchio, C. R., Alagna, F., Perniola, R., Bergamini, C., Rotunno, S., Calabrese, F. M., Crupi, P., Antonacci, M., Ventura, M. & Cardone, M. F. (2019). Transcriptomic and genomic structural variation analyses on grape cultivars reveal new insights into the genotype-dependent responses to water stress. *Scientific reports*, 9(1), 1-15.
- Chaves, M. M., Santos, T. P., Souza, C. D., Ortuño, M. F., Rodrigues, M. L., Lopes, C. M., Maroco, J. S. & Pereira, J. S. (2007). Deficit irrigation in grapevine improves water-use efficiency while controlling vigour and production quality. *Annals of applied biology*, 150(2), 237-252.
- Chaves, M. M., Zarrouk, O., Francisco, R., Costa, J. M., Santos, T., Regalado, A. P., Rodrigues, M.L. & Lopes, C. M. (2010). Grapevine under deficit irrigation: hints from physiological and molecular data. *Annals of botany*, 105(5), 661-676.
- Cifre, J., Bota, J., Escalona, J. M., Medrano, H., & Flexas, J. (2005). Physiological tools for irrigation scheduling in grapevine (*Vitis vinifera* L.): An open gate to improve water-use efficiency?. *Agriculture, Ecosystems & Environment*, 106(2-3), 159-170.
- Condon, A.G., Richards, R.A., Rebetzke, G.J. & Farquhar, G.D. (2004). Breeding for high water-use efficiency. *Journal of Experimental Botany* 55, 2447–2460.
- Corso M., Vannozzi, A., Maza, E., Vitulo, N., Meggio, F., Pitacco, A., Telatin, A., D'Angelo, M., Feltrin, E., Negri, A.S., Prinsi, B., Valle, G., Ramina, A., Bouzayen, M., Bonghi, C. & Lucchin, M. (2015). Comprehensive transcript profiling of two grapevine

rootstock genotypes contrasting in drought susceptibility links the phenylpropanoid pathway to enhanced tolerance. *Journal of Experimental Botany*, 66: 5739–52.

Costa, J.M., Ortuño, M.F., Lopes, C.M. & Chaves, M.M. (2012). Grapevine varieties exhibiting differences in stomatal response to water deficit. *Functional plant biology*, 39:179–189.

Costa, J.M., Vaz, M., Escalona, J.M., Egipto, R., Lopes, C., Medrano, H. & Chaves, M. M. (2016). Modern viticulture in southern Europe: vulnerabilities and strategies for adaptation to water scarcity. *Agricultural Water Management* 164:5-18

Costa, J. M., Vaz, M., Escalona, J. M., Egipto, R., Lopes, C. M., Medrano, H., & Chaves, M. M. (2020). Water as a critical issue for viticulture in southern Europe: sustainability vs competitiveness: Sourced from the research article “Modern viticulture in southern Europe: vulnerabilities and strategies for adaptation to water scarcity” (*Agricultural Water Management*, 2016). Original language of the article: English. IVES Technical Reviews, vine and wine.

Dayer, S., Herrera, J. C., Dai, Z., Burlett, R., Lamarque, L. J., Delzon, S. & Gambetta, G. A. (2020). The sequence and thresholds of leaf hydraulic traits underlying grapevine varietal differences in drought tolerance. *Journal of Experimental Botany*, 71(14), 4333-4344.

De Orduna, R. M. (2010). Climate change associated effects on grape and wine quality and production. *Food Research International*, 43(7), 1844-1855.

Deluc, L.G., Quilici, D.R., Decendit, A., Grimplet, J., Wheatley, M.D., Schlauch, K.A., Merillon, J.M., Cushman, J.C. & Cramer, G.R. (2009). Water deficit alters differentially metabolic pathways affecting important flavor and quality traits in grape berries of Cabernet Sauvignon and Chardonnay. *BMC Genomics*, 10(1), 1-33.

Douthe, C., Medrano, H., Tortosa, I., Escalona, J. M., Hernández-Montes, E., & Pou, A. (2018). Whole-plant water use in field grown grapevine: Seasonal and environmental effects on water and carbon balance. *Frontiers in plant science*, 9, 1540.

- Duchêne, E., & Schneider, C. (2005). Grapevine and climatic changes: a glance at the situation in Alsace. *Agronomy for sustainable development*, 25(1), 93-99.
- Düring, H. (2016). Evidence for osmotic adjustment to drought in grapevines (*Vitis vinifera* L.). *VITIS-Journal of Grapevine Research*, 23(1), 1.
- Eibach, R., & Töpfer, R. (2015). Traditional grapevine breeding techniques. In: *Grapevine breeding programs for the wine industry*. Woodhead Publishing, Oxford, pp 3-22.
- El Aou-ouad, H., Bota, J., Obata, T., Montero, R., Fernie, A. R., Medrano, H., & Florez-Sarasa, I. (2018). Combined drought and virus infection trigger aspects of respiratory metabolism related to grapevine physiological responses. *Journal of plant physiology*, 231, 19-30.
- Escalona, J.M., Flexas, J., Bota, J. and Medrano, H. (2003). Distribution of leaf photosynthesis and transpiration within grapevine canopies under different drought conditions. *Vitis*, 42,(2): 57-64.
- Escalona, J. M., Pou, A., Tortosa, I., Hernández-Montes, E., Tomás, M., Martorell, S., & Medrano, H. (2016). Using whole-plant chambers to estimate carbon and water fluxes in field-grown grapevines. *Theoretical and Experimental Plant Physiology*, 28(2), 241-254.
- Esperón-Rodríguez, M., Curran, T. J., Camac, J. S., Hofmann, R. W., Correa-Metrio, A., & Barradas, V. L. (2018). Correlation of drought traits and the predictability of osmotic potential at full leaf turgor in vegetation from New Zealand. *Austral ecology*, 43(4), 397-408.
- Farquhar, G. D. (1980). Carbon isotope discrimination by plants and the ratio of intercellular and atmospheric CO₂ concentrations. *Carbon Dioxide and Climate: Australian Research*, 105-110.
- Farquhar, G.D. & Richards, R. A. (1984). Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Australian Journal of Plant Physiology*, 11, 539–552.

- Farquhar, G. D., Von Caemmerer, S., & Berry, J. A. (2001). Models of photosynthesis. *Plant physiology*, 125(1), 42-45.
- Ferreira, I. Q., Rodrigues, M. Â., Moutinho-Pereira, J. M., Correia, C. M., & Arrobas, M. (2018). Olive tree response to applied phosphorus in field and pot experiments. *Scientia Horticulturae*, 234, 236-244.
- Flexas, J., & Medrano, H. (2002). Drought-inhibition of photosynthesis in C3 plants: stomatal and non-stomatal limitations revisited. *Annals of botany*, 89(2), 183-189.
- Flexas, J., Barón, M., Bota, J., Ducruet, J. M., Gallé, A., Galmés, J., Galmés J., Jiménez, M., Pou, A., Ribas-Carbó, M., Sajnami, C., Tomàs, M. & Medrano, H. (2009). Photosynthesis limitations during water stress acclimation and recovery in the drought-adapted *Vitis* hybrid Richter-110 (*V. berlandieri* × *V. rupestris*). *Journal of experimental Botany*, 60(8), 2361-2377.
- Flexas, J., Galmés, J., Gallé, A., Gulías, J., Pou, A., Ribas-Carbo, M., Tomàs & Medrano, H. (2010). Improving water use efficiency in grapevines: potential physiological targets for biotechnological improvement. *Australian Journal of Grape and Wine Research*, 16, 106-121.
- Florez-Sarasa, I., Clemente-Moreno, M. J., Cifre, J., Capó, M., Llompарт, M., Fernie, A. R., & Bota, J. (2020). Differences in Metabolic and Physiological Responses between Local and Widespread Grapevine Cultivars under Water Deficit Stress. *Agronomy*, 10(7), 1052.
- Fraga, H., & Santos, J. A. (2018). Vineyard mulching as a climate change adaptation measure: Future simulations for Alentejo, Portugal. *Agricultural systems*, 164, 107-115.
- Galmés, J., Molins, A., Flexas, J., & Conesa, M. À. (2017). Coordination between leaf CO₂ diffusion and Rubisco properties allows maximizing photosynthetic efficiency in *Limonium* species. *Plant, Cell & Environment*, 40(10), 2081-2094.
- Gambetta, G.A., Herrera, J.C., Dayer, S., Feng, Q., Hochberg, U. & Castellarin, S.D. (2020). The physiology of drought stress in grapevine: Towards an integrative definition of drought tolerance. *Journal of Experimental Botany*, 71, 4658–4676.

- Gauthier, A., Cookson, S. J., Lagalle, L., Ollat, N., & Marguerit, E. (2020). Influence of the three main genetic backgrounds of grapevine rootstocks on petiolar nutrient concentrations of the scion, with a focus on phosphorus. *OENO One*, 54(1), 1-13.
- Gil, P. M., Lobos, P., Duran, K., Olguin, J., Cea, D., & Schaffer, B. (2018). Partial root-zone drying irrigation, shading, or mulching effects on water savings, productivity and quality of 'Syrah' grapevines. *Scientia Horticulturae*, 240, 478-483.
- Gonzalez de Andres, E., Camarero, J. J., Blanco, J. A., Imbert, J. B., Lo, Y. H., Sangüesa-Barreda, G., & Castillo, F. J. (2018). Tree-to-tree competition in mixed European beech–Scots pine forests has different impacts on growth and water-use efficiency depending on site conditions. *Journal of Ecology*, 106(1), 59-75.
- Haider, M. S., Zhang, C., Kurjogi, M. M., Pervaiz, T., Zheng, T., Zhang, C., Lide C, Shanguan, L. & Fang, J. (2017). Insights into grapevine defense response against drought as revealed by biochemical, physiological and RNA-Seq analysis. *Scientific reports*, 7(1), 1-15.
- Han, J., Lei, Z., Flexas, J., Zhang, Y., Carriquí, M., Zhang, W., & Zhang, Y. (2018). Mesophyll conductance in cotton bracts: anatomically determined internal CO₂ diffusion constraints on photosynthesis. *Journal of Experimental Botany*, 69(22), 5433-5443.
- Hannah, L., Roehrdanz, P.R., Ikegami, M., Shepard, A.V., Shaw, M.R., Tabor, G., Zhi, L., Marquet, P.A., Hijmans, R.J. (2013) Climate change, wine, and conservation. *Proc Natl Acad Sci USA*, 110 (17), 6907–6912.
- Hernández-Montes, E. (2017). Respiratory processes and carbon balance in grapevines: environmental and genotype effects. Doctoral Thesis. University of Balearic Islands. Spain
- Hernández-Montes, E., Tomás, M., Escalona, J. M., Bota, J., & Medrano, H. (2019). Leaf growth rate and nitrogen content determine respiratory costs during leaf expansion in grapevines. *Physiologia plantarum*, 165(4), 746-754.
- Hochberg, U., Bonel, A. G., David-Schwartz, R., Degu, A., Fait, A., Cochard, H., Peterlunger, E. & Herrera, J. C. (2017). Grapevine acclimation to water deficit: the

- adjustment of stomatal and hydraulic conductance differs from petiole embolism vulnerability. *Planta*, 245(6), 1091-1104.
- Hochberg, U., Degu, A., Cramer, G. R., Rachmilevitch, S., & Fait, A. (2015). Cultivar specific metabolic changes in grapevines berry skins in relation to deficit irrigation and hydraulic behavior. *Plant Physiology and Biochemistry*, 88, 42-52.
- Hochberg, U., Rockwell, F. E., Holbrook, N. M., & Cochard, H. (2018). Iso/anisohdry: a plant–environment interaction rather than a simple hydraulic trait. *Trends in Plant Science*, 23(2), 112-120.
- Ibáñez, J., Carreño, J., Yuste, J., & Martínez-Zapater, J. M. (2015). Grapevine breeding and clonal selection programmes in Spain. In: *Grapevine breeding programs for the wine industry*, Woodhead Publishing, Oxford, pp 183–209
- Ibarrola-Rivas, M. J., Granados-Ramírez, R., & Nonhebel, S. (2017). Is the available cropland and water enough for food demand? A global perspective of the Land-Water-Food nexus. *Advances in Water Resources*, 110, 476-483.
- IPCC (2021) AR6 climate change 2021: impacts, adaptation and vulnerability — IPCC. In: IPCC <https://www.ipcc.ch/report/sixth-assessment-report-working-group-ii/>
- Jones, H.G. & Vaughan, R.A. (2010) *Remote sensing of vegetation: principles, techniques, and applications*. Oxford University Press, Oxford.
- Kaiser, E., Morales, A., Harbinson, J., Kromdijk, J., Heuvelink, E., & Marcelis, L. F. (2015). Dynamic photosynthesis in different environmental conditions. *Journal of Experimental Botany*, 66(9), 2415-2426.
- Leakey, A. D., Ferguson, J. N., Pignon, C. P., Wu, A., Jin, Z., Hammer, G. L., & Lobell, D. B. (2019). Water use efficiency as a constraint and target for improving the resilience and productivity of C3 and C4 crops. *Annual Review of Plant Biology*, 70, 781-808.
- Levin, A. D., & Kc, A. N. (2020). Water Deficits Do Not Improve Fruit Quality in Grapevine Red Blotch Virus-Infected Grapevines (*Vitis vinifera* L.). *Frontiers in Plant Science*, 11, 1292.

- Linares Torres, R., De La Fuente Lloreda, M., Junquera Gonzalez, P., Lissarrague García-Gutierrez, J. R., & Baeza Trujillo, P. (2018). Effect of soil management strategies on the characteristics of the grapevine root system in irrigated vineyards under semi-arid conditions. *Australian journal of grape and wine research*, 24(4), 439-449.
- Liu, B., Liang, J., Tang, G., Wang, X., Liu, F., & Zhao, D. (2019). Drought stress affects on growth, water use efficiency, gas exchange and chlorophyll fluorescence of *Juglans* rootstocks. *Scientia horticulturae*, 250, 230-235.
- López-Urrea, R., Montoro, A., Mañas, F., López-Fuster, P., & Fereres, E. (2012). Evapotranspiration and crop coefficients from lysimeter measurements of mature 'Tempranillo' wine grapes. *Agricultural water management*, 112, 13-20.
- Lovisolò, C., Perrone, I., Carra, A., Ferrandino, A., Flexas, J., Medrano, H., & Schubert, A. (2010). Drought-induced changes in development and function of grapevine (*Vitis* spp.) organs and in their hydraulic and non-hydraulic interactions at the whole-plant level: a physiological and molecular update. *Functional plant biology*, 37(2), 98-116.
- Ma, X., Sanguinet, K. A., & Jacoby, P. W. (2020). Direct root-zone irrigation outperforms surface drip irrigation for grape yield and crop water use efficiency while restricting root growth. *Agricultural Water Management*, 231, 105993.
- Martínez-García, P. J., Hartung, J., Pérez de los Cobos, F., Martínez-García, P., Jalili, S., Sánchez-Roldán, J. M., Rubio, M. Dicenta, F. & Martínez-Gómez, P. (2020). Temporal response to drought stress in several *Prunus* rootstocks and wild species. *Agronomy*, 10(9), 1383.
- Martorell, S., Medrano, H., Tomàs, M., Escalona, J. M., Flexas, J., & Diaz-Espejo, A. (2015a). Plasticity of vulnerability to leaf hydraulic dysfunction during acclimation to drought in grapevines: an osmotic-mediated process. *Physiologia plantarum*, 153(3), 381-391.
- Martorell, S., Diaz-Espejo, A., Tomàs, M., Pou, A., El Aou-ouad, H., Escalona, J. M., Vadell, J., Ribas-Carbó, M., Flexas, J. & Medrano, H. (2015b). Differences in water-use-efficiency between two *Vitis vinifera* cultivars (Grenache and Tempranillo) explained by

the combined response of stomata to hydraulic and chemical signals during water stress. *Agricultural Water Management*, 156, 1-9.

Marusig, D., & Tombesi, S. (2020). Abscisic acid mediates drought and salt stress responses in *vitis vinifera*—A review. *International Journal of Molecular Sciences*, 21(22), 8648.

Mathobo, R., Marais, D., & Steyn, J. M. (2017). The effect of drought stress on yield, leaf gaseous exchange and chlorophyll fluorescence of dry beans (*Phaseolus vulgaris* L.). *Agricultural Water Management*, 180, 118-125.

Medrano, H., Escalona, J. M., Bota, J., Gulías, J., & Flexas, J. (2002). Regulation of photosynthesis of C3 plants in response to progressive drought: stomatal conductance as a reference parameter. *Annals of botany*, 89(7), 895-905.

Medrano, H., Escalona, J. M., Cifre, J., Bota, J., & Flexas, J. (2003). A ten-year study on the physiology of two Spanish grapevine cultivars under field conditions: effects of water availability from leaf photosynthesis to grape yield and quality. *Functional Plant Biology*, 30(6), 607-619.

Medrano, H., Flexas, J., Ribas-Carbó, M. and Gulías, J. (2010) Measuring water use efficiency in grapevines. In *Methodologies and Results in Grapevine Research*. 123-134. Ed Springer.

Medrano, H., Pou, A., Tomás, M., Martorell, S., Gulias, J., Flexas, J., Escalona J.M. (2012) Average daily light interception determines leaf water use efficiency among different canopy locations in grapevine. *Agricultural Water Management*, 114: 4-10.

Medrano, H., Tomàs, M., Martorell, S., Escalona, J.M., Pou, A., Fuentes, S., Flexas, J., Bota, J. (2015a). Improving water use efficiency of vineyards in semi-arid regions. A review. *Agronomy For Sustainable Development*, 35, 499-517.

Medrano, H., Tomás, M., Martorell, S., Flexas, J., Hernández, E., Rosselló, J., Pou, A., Escalona, J.M. and Bota, J. (2015b) From leaf to whole plant water use efficiency (WUE) in complex canopies: Limitations of leaf WUE as selection target. *Crop journal*, 3, 220-228.

- Migicovsky, Z., Cousins, P., Jordan, L. M., Myles, S., Striegler, R. K., Verdegaal, P., & Chitwood, D. H. (2021). Grapevine rootstocks affect growth-related scion phenotypes. *Plant direct*, 5(5), 320-324.
- Mozell, M. R., & Thach, L. (2014). The impact of climate change on the global wine industry: Challenges & solutions. *Wine Economics and Policy*, 3(2), 81-89.
- Muñoz, C., Gomez-Talquenca, S., Chialva, C., Ibáñez, J., Martinez-Zapater, J. M., Peña-Neira, A., & Lijavetzky, D. (2014). Relationships among gene expression and anthocyanin composition of Malbec grapevine clones. *Journal of agricultural and food chemistry*, 62(28), 6716-6725.
- Muscas, E., Cocco, A., Mercenaro, L., Cabras, M., Lentini, A., Porqueddu, C., & Nieddu, G. (2017). Effects of vineyard floor cover crops on grapevine vigor, yield, and fruit quality, and the development of the vine mealybug under a Mediterranean climate. *Agriculture, Ecosystems & Environment*, 237, 203-212.
- Nadal, M., Flexas, J., & Gulias, J. (2018). Possible link between photosynthesis and leaf modulus of elasticity among vascular plants: a new player in leaf traits relationships? *Ecology letters*, 21(9), 1372-1379.
- Naulleau, A., Gary, C., Prévot, L., & Hossard, L. (2021). Evaluating Strategies for Adaptation to Climate Change in Grapevine Production—A Systematic Review. *Frontiers in Plant Science*, 11, 2154.
- Niinemets, Ü., Wright, I. J., & Evans, J. R. (2009). Leaf mesophyll diffusion conductance in 35 Australian sclerophylls covering a broad range of foliage structural and physiological variation. *Journal of Experimental Botany*, 60(8), 2433-2449.
- OIV (2019). OIV process for the recovery and conservation of the intravarietal diversity and the polyclonal selection of the vine in grape varieties with wide genetic variability. <https://www.oiv.int/public/medias/6939/oiv-viti-564b-2019-en.pdf>.
- Ollat, N., Cookson, S. J., Lauvergeat, V., Marguerit, E., Barrieu, F., Gambetta, G., Goutouly, J.P., Tandonnet, P., Vivin, P. & Delrot, S. (2016). Grapevine roots: the dark

side. In X International Symposium on Grapevine Physiology and Biotechnology 1188 (pp. 213-226).

Opazo, I., Toro, G., Solis, S., Salvatierra, A., Franck, N., Albornoz, F., & Pimentel, P. (2019). Late reduction on transpiration is an important trait for water deficit tolerance in interspecific *Prunus* rootstock hybrids. *Theoretical and Experimental Plant Physiology*, 31(4), 493-506.

Patakas, A., Stavrakas, D., & Fisarakis, I. (2003). Relationship between CO₂ assimilation and leaf anatomical characteristics of two grapevine cultivars. *Agronomie*, 23(4), 293-296.

Peña, J.P. & Tarara, J.M. (2004). A portable whole canopy gas exchange system for several mature field-grown grapevines. *Vitis-Geilweilerhof*, 43: 7-14.

Poni, S., Bernizzoni, F., Civardi, S., Gatti, M., Porro, D. and Camin, F. (2009). Performance and water-use efficiency (single-leaf vs. whole-canopy) of well-watered and half-stressed split-root Lambrusco grapevines grown in Po Valley (Italy). *Agriculture, Ecosystems & Environment* 129, 97-106.

Poni, S., Merli, M.C., Magnanini, E., Galbignani, M., Bernizzoni, F., Vercesi, A. and Gatti, M. (2014). An Improved multi chamber gas exchange system for determining whole-canopy water-use *Am. J. Enol. Vitic.* 65:2 268-276.

Poorter, H., Bühler, J., van Dusschoten, D., Climent, J., & Postma, J. A. (2012). Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. *Functional Plant Biology*, 39(11), 839-850.

Prieto, J. A., Lebon, E., & Ojeda, H. (2010). Stomatal behavior of different grapevine cultivars in response to soil water status and air water vapor pressure deficit. *International Viticulture and Enology Society (IVES)*.

Prieto, J. A., Louarn, G., Perez Pena, J., Ojeda, H., Simonneau, T., & Lebon, E. (2012). A leaf gas exchange model that accounts for intra-canopy variability by considering leaf nitrogen content and local acclimation to radiation in grapevine (*Vitis vinifera* L.). *Plant, cell & environment*, 35(7), 1313-1328.

- Ramu, P., Esuma, W., Kawuki, R., Rabbi, I. Y., Egesi, C., Bredeson, J. V., Bart, R. S., Verma, J., Buckler, E. S. & Lu, F. (2017). Cassava haplotype map highlights fixation of deleterious mutations during clonal propagation. *Nature Genetics*, 49(6), 959-963.
- Resco de Dios, V. (2017). Circadian regulation and diurnal variation in gas exchange. *Plant physiology*, 175(1), 3-4.
- Riffle, V., Palmer, N., Casassa, L. F., & Dodson Peterson, J. C. (2021). The Effect of Grapevine Age (*Vitis vinifera* L. cv. Zinfandel) on Phenology and Gas Exchange Parameters over Consecutive Growing Seasons. *Plants*, 10(2), 311.
- Rocheta, M., Coito, J. L., Ramos, M. J., Carvalho, L., Becker, J. D., Carbonell-Bejerano, P., & Amâncio, S. (2016). Transcriptomic comparison between two *Vitis vinifera* L. varieties (Trincadeira and Touriga Nacional) in abiotic stress conditions. *BMC plant biology*, 16(1), 1-19.
- Rodríguez-Gamir, J., Xue, J., Meason, D. F., Clearwater, M., Clinton, P. W., & Domec, J. C. (2021). Interclonal variation, coordination, and trade-offs between hydraulic conductance and gas exchange in *Pinus radiata*: consequences on plant growth and wood density. *Journal of Experimental Botany*, 72(7), 2419-2433.
- Roig-Oliver, M., Nadal, M., Clemente-Moreno, M. J., Bota, J., & Flexas, J. (2020). Cell wall components regulate photosynthesis and leaf water relations of *Vitis vinifera* cv. Grenache acclimated to contrasting environmental conditions. *Journal of plant physiology*, 244, 153084.
- Romero, P., Muñoz, R. G., Fernández-Fernández, J. I., del Amor, F. M., Martínez-Cutillas, A., & García-García, J. (2015). Improvement of yield and grape and wine composition in field-grown Monastrell grapevines by partial root zone irrigation, in comparison with regulated deficit irrigation. *Agricultural Water Management*, 149, 55-73.
- Romero, P., Botía, P., & Navarro, J. M. (2018). Selecting rootstocks to improve vine performance and vineyard sustainability in deficit irrigated Monastrell grapevines under semiarid conditions. *Agricultural Water Management*, 209, 73-93.

- Romero, P., Botía, P., del Amor, F. M., Gil-Muñoz, R., Flores, P., & Navarro, J. M. (2019). Interactive effects of the rootstock and the deficit irrigation technique on wine composition, nutraceutical potential, aromatic profile, and sensory attributes under semiarid and water limiting conditions. *Agricultural Water Management*, 225, 105733.
- Rühl, E., Konrad, H., Lindner, B., & Bleser, E. (2003). Quality criteria and targets for clonal selection in grapevine. In: *I International Symposium on Grapevine Growing, Commerce and Research 652* (pp. 29-33).
- Sadras, V. O. (2009). Does partial root-zone drying improve irrigation water productivity in the field? A meta-analysis. *Irrigation Science*, 27(3), 183-190.
- Santesteban, L. G., Miranda, C., Barbarin, I., & Royo, J. B. (2015). Application of the measurement of the natural abundance of stable isotopes in viticulture: a review. *Australian journal of grape and wine research*, 21(2), 157-167.
- Santillán, D., Iglesias, A., La Jeunesse, I., Garrote, L., & Sotes, V. (2019). Vineyards in transition: A global assessment of the adaptation needs of grape producing regions under climate change. *Science of the Total Environment*, 657, 839-852.
- Santos, E. F., Mateus, N. S., Rosario, M. O., Garcez, T. B., Mazzafera, P., & Lavres, J. (2021). Enhancing potassium content in leaves and stems improves drought tolerance of eucalyptus clones. *Physiologia Plantarum*, 172(2), 552-563.
- Sepaskhah, A. R., & Ahmadi, S. H. (2012). A review on partial root-zone drying irrigation. *International Journal of Plant Production*, 4(4), 241-258.
- Shahmoradi, M., Shekafandeh, A., & Eshghi, S. (2019). Physiological and Biochemical Changes of Some Grapevine Cultivars under Different Irrigation Regimes. *Agriculturae Conspectus Scientificus*, 84(4), 371-382.
- Sharma, A., Wang, J., Xu, D., Tao, S., Chong, S., Yan, D., Li Z., Yuan, H. & Zheng, B. (2020). Melatonin regulates the functional components of photosynthesis, antioxidant system, gene expression, and metabolic pathways to induce drought resistance in grafted *Carya cathayensis* plants. *Science of the Total Environment*, 713, 136675.

- Soar, C. J., Dry, P. R., & Loveys, B. R. (2006). Scion photosynthesis and leaf gas exchange in *Vitis vinifera* L. cv. Shiraz: mediation of rootstock effects via xylem sap ABA. *Australian Journal of Grape and Wine Research*, 12(2), 82-96.
- Tello, J., Montemayor, M. I., Forneck, A., & Ibáñez, J. (2018). A new image-based tool for the high throughput phenotyping of pollen viability: evaluation of inter-and intra-cultivar diversity in grapevine. *Plant methods*, 14(1), 1-17.
- This P., Lacombe T., Thomas M. R. (2006). Historical origins and genetic diversity of wine grapes. *Trends Genet*, 22: 511-519
- Tilman, D., Balzer, C., Hill, J., & Befort, B. L. (2011). Global food demand and the sustainable intensification of agriculture. *Proceedings of the national academy of sciences*, 108(50), 20260-20264.
- Tomás, M., Medrano, H., Pou, A., Escalona, J. M., Martorell, S., Ribas-Carbó, M., & Flexas, J. (2012). Water-use efficiency in grapevine cultivars grown under controlled conditions: effects of water stress at the leaf and whole-plant level. *Australian Journal of Grape and Wine Research*, 18(2), 164-172.
- Tomàs, M., Medrano, H., Escalona, JM., Martorell, S., Pou, A., Ribas-Carbó, M., Flexas J. (2014a). Variability of water use efficiency in grapevines *Environmental and Experimental Botany* 103: 148–157.
- Tomàs, M., Medrano, H., Brugnoli, E., Escalona, J.M., Martorell, S., Pou, A.; Ribas-Carbó, M., Flexas, J. (2014b). Variability of mesophyll conductance in grapevines cultivars under water stress conditions in relation to leaf anatomy and water use efficiency. *Australian Journal of Grape and Wine Research* 20: 272- 280.
- Tortosa, I., Escalona, J. M., Bota, J., Tomas, M., Hernandez, E., Escudero, E. G., & Medrano, H. (2016). Exploring the genetic variability in water use efficiency: evaluation of inter and intra cultivar genetic diversity in grapevines. *Plant Science*, 251, 35-43.

- Tortosa, I., Douthe, C., Pou, A., Balda, P., Hernandez-Montes, E., Toro, G., Escalona, J.M. & Medrano, H. (2019a). Variability in water use efficiency of grapevine Tempranillo clones and stability over years at field conditions. *Agronomy*, 9(11), 701.
- Tortosa, I., Escalona, J. M., Douthe, C., Pou, A., Garcia-Escudero, E., Toro, G., & Medrano, H. (2019b). The intra-cultivar variability on water use efficiency at different water status as a target selection in grapevine: Influence of ambient and genotype. *Agricultural Water Management*, 223, 105648.
- Tortosa, I., Escalona, J. M., Toro, G., Douthe, C., & Medrano, H. (2020). Clonal behavior in response to soil water availability in Tempranillo grapevine cv: from plant growth to water use efficiency. *Agronomy*, 10(6), 862.
- van Leeuwen, C., Pieri, P., Gowdy, M., Ollat, N., & Roby, J. P. (2019). Reduced density is an environmental friendly and cost effective solution to increase resilience to drought in vineyards in a contexte of climate change. *Oeno One*, 53(2), 129-146.
- Vondras, A. M., Minio, A., Blanco-Ulate, B., Figueroa-Balderas, R., Penn, M. A., Zhou, Y., Seymour, D., Ye, Z., Liang, D., Espinoza, L. K., Anderson, M.M. Walker, M. A., Gaut, B. & Cantu, D. (2019). The genomic diversification of grapevine clones. *BMC genomics*, 20(1), 1-19.
- Wang, Y. X., Jia, X. M., Shi, X. Y., Zhu, Y. F., HU, Y., & GUO, A. X. (2018). The response characteristics of the ultrastructure and anatomical structure of three apple rootstocks under drought stress. *Plant Physiol*, 54, 94-606.
- Wenter, A., Zanutelli, D., Montagnani, L., Tagliavini, M., & Andreotti, C. (2018). Effect of different timings and intensities of water stress on yield and berry composition of grapevine (cv. Sauvignon blanc) in a mountain environment. *Scientia Horticulturae*, 236, 137-145.
- Williams, M. R., Coronel, O., McAfee, S. J., & Sanders, L. L. (2020). Preferential flow of surface-applied solutes: Effect of lysimeter design and initial soil water content. *Vadose Zone Journal*, 19(1), e20052.

Zhang, L., Marguerit, E., Rossdeutsch, L., Ollat, N., & Gambetta, G. A. (2016). The influence of grapevine rootstocks on scion growth and drought resistance. *Theoretical and Experimental Plant Physiology*, 28(2), 143-157.

Zhou, H., Xu, M., Hou, R., Zheng, Y., Chi, Y., & Ouyang, Z. (2018). Thermal acclimation of photosynthesis to experimental warming is season-dependent for winter wheat (*Triticum aestivum* L.). *Environmental and Experimental Botany*, 150, 249-259.

Zhao, W., Liu, L., Shen, Q., Yang, J., Han, X., Tian, F., & Wu, J. (2020). Effects of water stress on photosynthesis, yield, and water use efficiency in winter wheat. *Water*, 12(8), 2127.

Zufferey, V., Verdenal, T., Dienes, A., Belcher, S., Lorenzini, F., Koestel, C. & Spring, J. L. (2018). The impact of plant water status on the gas exchange, berry composition and wine quality of Chasselas grapes in Switzerland: Impacts of water stress on grapevine physiology. *Oeno One*, 52(4).