Universitat de les Illes Balears

Genetic variability in a grapevine progeny (*Vitis vinifera* L. cvs Grenache × Syrah) using reduced night-time transpiration as the target parameter

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ABSTRACT

Grapevine is one of the most important crops worldwide, but its sustainability is threatened by climate change. The rise in temperature and the decrease in rainfall reduce water availability in the Mediterranean region and plants experiment higher transpiration rates due to the planet warming. Hence, this study focuses in water relations and transpiration in plants, especially at night. Previous researchers found genetic differences in cultivar's stomatal control (they show an isohydric or an anisohydric behaviour), then the cross between two cultivars with different behaviours results in a progeny with a high genetic variability. The aim was to identify genotypes with reduced night-time transpiration paired with high photosynthetic taxes in order to maintain the grapevine sustainability in the Mediterranean region in a climate change scenario.

Keywords

Vitis vinifera, night-time transpiration, genetic variability, progeny, water use efficiency.

INTRODUCTION

Grapevine is one of the most important crops worldwide, mainly because of the importance of its final products (Vivier and Pretorius, 2002). The most used species is the European grapevine (*Vitis vinifera* L.), usually grafted on American varieties. Worldwide production is centered in few varieties with particular traits, both agronomic and wine-related (Torregrossa et al., 2014).

Climate change threatens crops sustainability due to summer rainfall reduction coupled to an increase in temperature and a drop-in soil humidity (Ragad and Prudhomme, 2002). The increased temperature implies a greater CO2 accumulation, and both reduce water availability, especially in temperate climates or the Mediterranean basin (Schultz and Stoll, 2010; Schultz, 2000), and it will be paired with more droughts in the future (Sheffield and Wood, 2008). Hence, one of the major growth regions for grapevines will be endangered by climate change and will no longer be sustainable for this crop (Hannah et al., 2013).

The CO_2 increase may be greater than expected due to organic matter degradation because of higher temperatures (Schultz and Stoll, 2010). CO_2 concentrations also affect the mesophyll conductance and may alter the photosynthetic efficiency (Flexas et al., 2007; Flexas et al., 2008). On one hand, higher CO_2 increase the photosynthetic tax and plant growth and production worldwide. On the other hand, the pairing with the CO_2 enrichment and temperature will cause a warming in plant surfaces and higher transpiration rates to regulate temperature (Schultz and Stoll, 2010). Moreover, most models do not consider this pairing and cannot provide a reasonable approach to the future conditions considering climate change. Previous studies indicated genetic differences in the CO_2 specificity factor of Rubisco, studied in plants in water deficit and well-watered conditions (Bota et al., 2001). Therefore, there is a need to search for efficient genotypes under greater CO_2 conditions.

There are differences among grapevine cultivars based in their stomatal control, and two behaviours have been described: isohydric and anisohydric (Schultz, 2003). Isohydric cultivars maintain a constant foliar water potential, regardless of soil moisture or the vapor pressure deficit. Anisohydric cultivars, however, lower their foliar water potential when the soil gets drier (Lovisolo et al., 2010; Sade et al., 2012). Thus, the cross between two cultivars with different behaviours results in a progeny with a high genetic variability (Coupel-Ledru et

al., 2014). In addition, previous studies support that more efficient plants should be the aim for future breeding programs (Coupel-Ledru et al., 2016).

Most regions with a Mediterranean climate have experienced significant reductions in rains, with a major effect in the South and increased water scarcity (del Río et al., 2010; Santos et al., 2010). If the situation persists, irrigation will be needed to support grapevine growth and production, but the economic profit and crop sustainability will decrease (Gonsier, 2015), as water is a limited resource. These are the reasons why reducing water use efficiency should be a top concern in the near future (Flexas at al., 2010).

Leaves must maintain a certain turgor pressure to function correctly, and they have various mechanisms to keep it constant when they are water-stressed. The plant leaf water potential (Ψ_l) is a measure of the plant water status and decreases as the soil moisture drops (Rogiers et al, 2011). Likewise, osmotic potential (Ψ_o) is also an indicator of the plant water status. The plants tend to accumulate more solutes to maintain cell turgor, so their metabolism is not affected by water scarcity (Düring, 1984; Rodrigues et al., 1993; Schultz and Matthews, 1993). As said by Martorell et al. (2014) an active osmotic adjustment by the plant leads to more negative water potentials that help the plant to absorb water from the soil and maintain the leaf turgor. Then, leaf water potential and osmotic potential through the day are also an indicator of the refilling capacity of the plant, as they decrease during the night when the plant recovers from the depletion during the daytime (Zeppel et al., 2014).

The reduction of water consume can be achieved by improving the water use by the crops. The water use efficiency (WUE) is the ratio between the carbon gains (kg of produced biomass or mol of assimilated CO2) and the water losses (m3 of water consumed or mol of transpired water). WUE can be measured at different scales, starting from the leaf level to the watershed level. At leaf level, the ratio between the net assimilation tax of CO2 (A_n) and stomatal conductance (g_s) represents the intrinsic water use efficiency (WUE_i). When dividing A_n among the leaf transpiration rate (E) the result is the instantaneous water use efficiency (WUE_{inst}) (Fischer and Turner, 1978; Morison et al., 2008). Scaling up, the crop water use efficiency (WUE_c) is obtained by dividing the plant production in kg by the water consumed (both the water used by the plant and the water that leaks into the soil or is evaporated)

(Medrano et al., 2015). To calculate the plant water use efficiency (WUE_p) gets difficult because there are no easy measures that represent the exact water transpired or the plant dry mass accumulation (Medrano et al., 2012). However, leaf-level measurements show a strong correlation to daily weighted values when the leaves are sun-exposed. Hence, leaf-level estimates are often used to compare and analyse differences among grapevine genotypes.

Most studies focus on decreasing stomatal conductance to improve WUE, as the stomata are the main channel of water losses at the leaf. Other targets are both improving photosynthesis and reducing carbon losses by interfering with plant respiration (Flexas et al., 2010). On one hand, the reduction of stomatal openness could imply a decrease in the carbon gain and the plant yield as it is coupled to photosynthesis during daytime. On the other hand, an interesting approach is the reduction of stomatal openness during night-time, as it does not interfere with carbon gain (Flexas et al., 2010; Medrano et al., 2012; Escalona et al., 2013). Recent studies show how stomatal conductance during night has an important role in the plant water losses due to night-time transpiration (Chu et al., 2009; Daley and Phillips, 2006; Rogiers et al., 2009). In grapevine, night-time transpiration can represent up to 10-20% of water losses during daytime (Escalona et al., 2013), but it can largely vary among cultivars or the chosen time to measure these parameters (pre-dawn values are usually higher than during the night, so estimates with pre-dawn measures are also higher). However, transpiration can be a target for choosing cultivars that are more efficient; in varieties with low night-time transpiration and high daytime transpiration, carbon gains during the day are not compromised by the water losses (Coupel-Ledru et al., 2016). Imposing a mild water deficit to grapevine reduces the transpiration rate (Coupel-Ledru et al., 2014), but this effect could be also lead by other parameters like the genetic variation found among a progeny. Moreover, the genetic variance can also be led by different rootstocks, which implies the need of further studies.

Objectives

The three main objectives of the present work are: (1) to study the genetic variability on night-time transpiration and photosynthetic rate in a progeny (*Vitis vinifera* L. cvs Grenache × Syrah) using grafted and ungrafted plants; (2) to study the genetic variability in a progeny (*Vitis vinifera* L. cvs Grenache × Syrah) under different CO_2 conditions and its implications in water use efficiency (WUE), and (3) to summarize the genotypes with reduced night-time transpiration paired with high photosynthetic taxes.

MATERIAL AND METHODS

Two main experiences were carried out in Montpellier (Supagro, Montpellier, France) from mid-July to mid-September 2017. The infrastructures used are the grapevine field, the PhenoDyn greenhouse and a culture chamber located in the Montpellier SupAgro campus, at the LEPSE (Laboratoire d'Écophysiologie des Plantes Sous Stress Environnementaux).

Experiment 1

Plant material and treatments

To study the behaviour of grafted and ungrafted plants towards night-time transpiration (objective 1), 83 three-year-old plants from 14 genotypes (12 crossed genotypes plus both parental cultivars, grafted onto 110-Richter rootstock; n=5-6) and 43 two-year-old-plants from 12 genotypes (10 crossed genotypes plus both parental cultivars, ungrafted; n=1-6) were transferred to the PhenoDyn greenhouse to record daytime and night-time transpiration (Table 1). Three year-old-plants were cultivated in 9 litres pots and 2 year-old-pots in 4,3 litres pots. For that purpose, plants were irrigated to soil field capacity (FC) every day. All pots were sealed with plastic bags for 48 hours to avoid water evaporation from the soil. Afterwards, plants were irrigated until they reached the target weight (FC) and sealed again with plastic bags for 24 hours.

Figure 1. PhenoDyn greenhouse with plants from experiment 1.

Before the experimented started in the greenhouse, grapes, ramifications longer than the main stem and tertiary ramifications of all plants were removed. Secondary ramifications which made the plant management or the foliar characterisation more difficult were also removed above the last fully expanded leaf. There was at least one secondary ramification not cut per plant to record stem elongation.

During a second phase of this experiment, three-year-old plants return to the experimental field and 36 two-year-old plants from 6 crossed genotypes (n=6) are transferred to PhenoDyn. They were more manageable and provided data to identify genotypes with reduced night-time transpiration. They were irrigated at field capacity and sealed with plastic bags for 24 hours, after that the automatic irrigation was scheduled twice a day until the soil reached field capacity.

Table 1. List of genotypes used in experiment 1. For the grafted column, "G" means grafted plants, and "UG" means ungrafted plants.

Cultivar code	**Transplant year**	**"Mother" plant**	**Grafted**
SYRAH	2013	Parental	G
GRENACHE	2013	Parental	G
7G031	2013	Grenache	G
7G036	2013	Grenache	G
7G039	2013	Grenache	G
7G068	2013	Grenache	G
7G092	2013	Grenache	G
7G096	2013	Grenache	G
8S002	2013	Syrah	G

8S012	2013	Syrah	G
8S034	2013	Syrah	G
8S037	2013	Syrah	G
8S051	2013	Syrah	G
8S092	2013	Syrah	G
SYRAH	2016	Parental	UG
GRENACHE	2016	Parental	UG
7G003	2016	Grenache	UG
7G031	2016	Grenache	UG
7G036	2016	Grenache	UG
7G039	2016	Grenache	UG
7G045	2016	Grenache	UG
7G046	2016	Grenache	UG
7G068	2016	Grenache	UG
7G092	2016	Grenache	UG
7G096	2016	Grenache	UG
8S002	2016	Syrah	UG
8S012	2016	Syrah	UG
8S034	2016	Syrah	UG
8S037	2016	Syrah	UG
8S040	2016	Syrah	UG

8S052	2016	Syrah	UG
8S092	2016	Syrah	UG

Field Climatic Conditions

The average temperature was 24,7ºC, with an air relative humidity which varied from 76% (night-time) to 46% (midday). The VPD in the PhenoDyn was maintained between 0,60 and 2,00 kPa, and temperature was maintained as close as possible to the outside conditions.

Foliar characterisation and total leaf surface

In the course of the experiment, leaf area of all 162 plants was estimated. Firstly, the total number of fully expanded leaves and the length of the two lateral nerves of at least a fifth of the total number of fully expanded leaves were recorded. For the secondary ramifications, only leaves in the same foliar stage were took into account and the lateral nerves of one or two leaves (if the ramification was longer and if there were more than 5 leaves) were measured. When there were several secondary ramifications, only the first three were considered for the foliar characterisation.

The following formulas from Phinopoulos et al. (2015) were used to estimate the single leaf area (LA, in cm^2), where V1 and V2 refer to the two lateral nerves:

$V1+V2=V2S$

$LA=e^{-6,436} \cdot V2S \cdot 2,167$

Then, with the area of the largest primary leaf (L_1) and the smallest primary leaf (S_1), the leaf area per shoot was estimated with the following formulas (Lopes and Pinto, 2005):

For the mean primary leaf area:

$M_1=(L_1+S_1)/2$

For the mean primary leaf area per shoot (MLA_1), where NL_1 refers to the number of leaves:

$LA_1=49,1936+0,9958 \cdot MLA_1$

Transpiration measurements

There are 168 scales in the PhenoDyn platform organized in 12 rows with 14 scales each one. Plants were randomly arranged on the scales, which recorded the weight every minute and submitted it to a database.

Experiment 2

Plant material and treatments

This experiment was designed to examine the connexion between night-time transpiration and physiological parameters (objectives 2 and 3). The experiment took place inside a culture chamber with 15 scales (Figure 2) with 10 genotypes (Table 2) (8 crossed genotypes plus both parental cultivars, n=3). Due to the culture chamber size, only 5 genotypes were inside at each time. Plants were irrigated twice a day with 300 ml to achieve field capacity. The photoperiod was stablished as close as possible to the photoperiod in the field:

- Daytime lasted 14,5 hours (from 6:30h to 21h), with an average temperature of 27°C and an average VPD of 2,4 kPa.
- Night-time lasted 9,5 hours (from 21h to 6:30h), with an average temperature of 21°C and an average VPD of 0,7 kPa.

Pots were sealed with plastic bags to record mainly night-time transpiration and dendrometers were also installed to record changes in stem diameter.

After the first two weeks, photoperiod was stablished with a several hours gap:

- Daytime lasted 14,5 hours (from 0:30h to 15h), with an average temperature of 27°C and an average VPD of 2,4 kPa.
- Night-time lasted 9,5 hours (from 15h to 0:30h), with an average temperature of 21°C and an average VPD of 0,7 kPa.

Table 2. List of genotypes used in experiment 2.

Cultivar code	**Transplant year**	**“Mother” plant**
SYRAH	2016	Parental
GRENACHE	2016	Parental
7G036	2016	Grenache

7G045	2016	Grenache
7G046	2016	Grenache
8S002	2016	Syrah
8S012	2016	Syrah
8S034	2016	Syrah
8S040	2016	Syrah
8S052	2016	Syrah

Figure 2. Culture chamber with plants from experiment 2.

Growing measurements

Before transferring plants to the culture chamber, it was measured the main stem length from the last fully expanded leaf to the apex, the total number of leaves and the lateral foliar nerves of these leaves. A plastic ring was placed after the last fully expanded leaf, so the measurements could be repeated when plants leave the chamber to record the total growth. In addition, one not fully expanded leaf per plant was selected to photograph daily or twice a day in order to compare daytime versus night-time growth and estimate the leaf surface growth with the following formula:

Leaf surface growth = (final LA - initial LA)/(initial LA) · 100

Transpiration Measurements

See Experiment 1.

Leaf gas exchange measurements

Leaf gas exchange measurements were performed under outdoors conditions with an open infrared gas-exchange analyser (Li-6400, Li-Cor Inc., Lincoln, USA) between 10h and 16h (local time) (Figure 3). The exposed leaf surface inside the chamber was 6cm2, with a leaf temperature of approximate 27°C, with photon flux density (PPFD) of 1800 μmol m^{-2} s^{-1}, a vapor pressure deficit of 1.74 ± 0.27 kPa and an air flow set at 400 μmol air min^{-1} at a CO_2 ambient concentration (C_a) of 400 μmol CO_2 mol^{-1} air, and at a CO_2 saturated concentration 1700 μmol CO_2 mol^{-1} air. Leaf net photosynthesis was measured in one fully exposed leaf per plant (n=3) for each of the 10 genotypes.

Figure 3. Process of taking leaf gas exchange measurements with an open infrared gas-exchange analyzer during experiment 2.

Leaf Water Potential and Osmotic Potential

The leaf water potential (Ψ_l) was measured with a Scholander pressure chamber (Soil moisture Equipment Corp. Santa Barbara, California USA). It was measured in one leaf per plant (n=3) during the first two hours of night-time.

Leaf osmotic potential (Ψ_o) was measured in 2 leaves per plant (n=6) which were preserved at -80°C. After defrosting, leaves were macerated in a mortar with a pestle. 50µl aliquots of the resultant sap were collected and centrifugated at 2000 g for 2 min at 4°C (methodology determined according preliminary tests). The osmotic potential was determined with a an osmometer (Vapro® Vapor Pressure Osmometer Model 5520, Wescor Inc., Logan, UT, EEUU) using 10µl aliquots. Measurements with the osmometer were repeated 3 times for each leaf.

RESULTS AND DISCUSSION

Genetic variability in night-time transpiration

Experiment 1 was designed to study the genetic variability in night-time transpiration in grafted (G) and ungrafted (UG) plants. The night-time transpiration (Tr_n) (Figure 4) and daytime transpiration (Tr_d) show significant differences between G and UG, as they differed in plant age and size (Figure 4). To be able to observe the genetic variability in these parameters, Tr_n measures were divided by total leaf area (LA, Table 3).

ANOVA did not reveal significant differences in the same genotype between the grafted (G) and ungrafted (UG) plants for the transpiration parameters (data not shown). However, it showed differences in Tr_n/LA (Table 3) in the following genotypes when comparing G with UG plants: Grenache, Syrah, 7G031, 7G036, 7G039, 7G068, 7G092, 7G096, 8S002, 8S012, 8S034, 8S037 and 8S092. In all cases, G plants had higher Tr_n/LA than UG ones, but G plants are also older than UG, and values ranged from 6,68 $\cdot 10^{-4}$ (8S002) and 4,6 $\cdot 10^{-4}$ l H_2O cm^{-2} (7G068) in G plants, and 8,07 $\cdot 10^{-4}$ (7G031) and 1,52 $\cdot 10^{-4} \pm 4,7 \cdot 10^{-5}$ l H_2O cm^{-2} (7G046) in UG plants. The genotype with the highest rate is 7G031, which is a G plant.

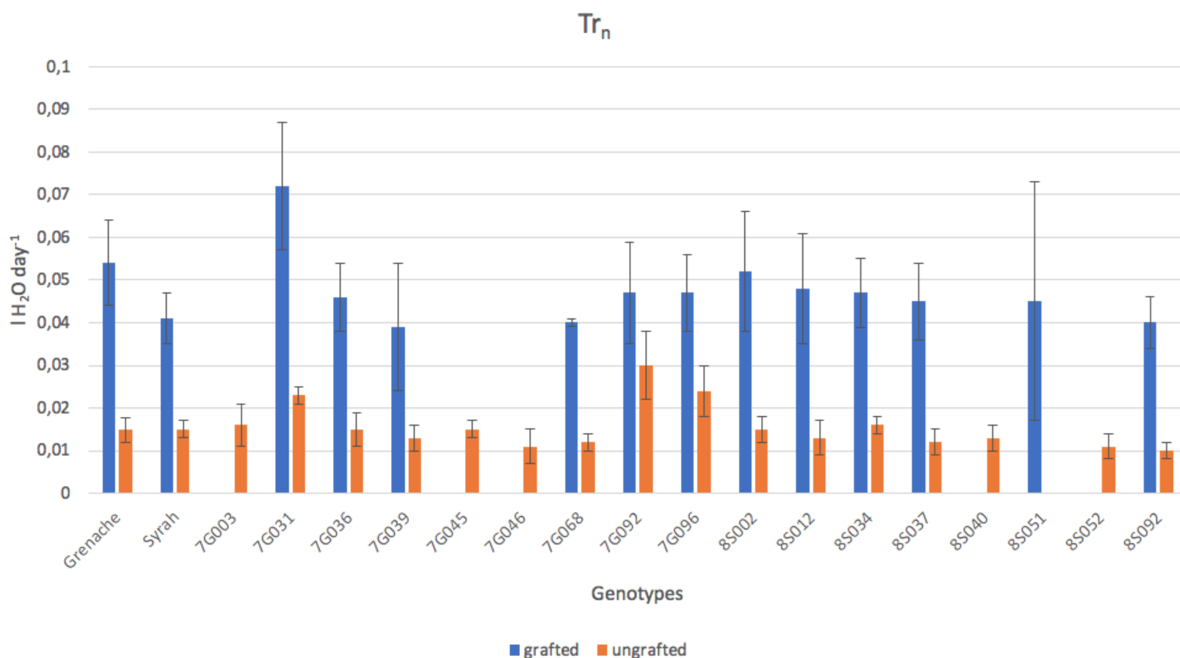

Figure 4. Variability in night-time transpiration (Tr_n, l H_2O day^{-1}) among the genotypes. Blue bars represent grafted plants (G) and orange bars represent ungrafted plants (UG).

Table 3. Variability in night-time transpiration divided by leaf area (Tr_n/LA ± standard error) among the genotypes. Letters indicate significant differences between different genotypes. In the grafted column, "G" represents that they are grafted and "UG" represents they are ungrafted.

Cultivar code	Transplant year	Tr_n/LA (l H_2O cm^{-2})	Grafted
SYRAH	2013	0,00049 ± 0,0001 bc	G
GRENACHE	2013	0,00058 ± 0,00012 bc	G
7G031	2013	0,00088 ± 0,00021 a	G
7G036	2013	0,00048 ± 0,0001 bc	G
7G039	2013	0,00051 ± 0,00023 bc	G
7G068	2013	0,00046 ± 0,0001 bc	G
7G092	2013	0,00057 ± 0,00015 bc	G
7G096	2013	0,00061 ± 0,00015 bc	G
8S002	2013	0,00067 ± 0,00025 ab	G
8S012	2013	0,00060 ± 0,00013 bc	G
8S034	2013	0,00055 ± 0,00013 bc	G
8S037	2013	0,00057 ± 0,00011 bc	G
8S051	2013	0,00057 ± 0,00041 bc	G
8S092	2013	0,00046 ± 0,00007 bc	G
SYRAH	2016	0,00024 ± 0,00003 de	UG
GRENACHE	2016	0,00021 ± 0,00005 e	UG
7G003	2016	0,00021 ± 0,00003 e	UG

7G031	2016	0,00033 ± 0,00004 cde	UG
7G036	2016	0,00023 ± 0,00007 de	UG
7G039	2016	0,00019 ± 0,00005 e	UG
7G045	2016	0,00022 ± 0,00003 e	UG
7G046	2016	0,00015 ± 0,00005 e	UG
7G068	2016	0,00020 ± 0,00004 e	UG
7G092	2016	0,00043 ± 0,00011 cd	UG
7G096	2016	0,00034 ± 0,00009 cde	UG
8S002	2016	0,00022 ± 0,00004 e	UG
8S012	2016	0,00019 ± 0,00006 e	UG
8S034	2016	0,00021 ± 0,00002 e	UG
8S037	2016	0,00017 ± 0,00002 e	UG
8S040	2016	0,00018 ± 0,00003 e	UG
8S052	2016	0,00017 ± 0,00004 e	UG
8S092	2016	0,00017 ± 0,00003 e	UG

However, when comparing genotypes with the same treatment or comparing ungrafted or grafted plants with their corresponding parentals, ANOVA did not reveal significant differences (Tables 4-5). Ungrafted plants values double the grafted plants results when comparing the data among the same treatment (Table 4).

Table 4. Variability in night-time transpiration (Tr_n ± standard error), daytime transpiration (Tr_d ± standard error) and night-time transpiration divided by leaf area (Tr_n/LA ± standard error) among the treatments. Letters indicate significant differences between different treatments.

	Ungrafted (UG)	**Grafted (G)**
Tr_n (l H_2O day^{-1})	$4{,}8 \cdot 10^{-2} \pm 1{,}5 \cdot 10^{-2}$ a	$1{,}5 \cdot 10^{-2} \pm 5{,}6 \cdot 10^{-3}$ b
Tr_d (l H_2O day^{-1})	$7{,}7 \cdot 10^{-1} \pm 1{,}6 \cdot 10^{-1}$ a	$2{,}6 \cdot 10^{-1} \pm 7{,}2 \cdot 10^{-2}$ b
Tr_n/LA (l H_2O cm^{-2})	$5{,}8 \cdot 10^{-4} \pm 2 \cdot 10^{-4}$ a	$2{,}2 \cdot 10^{-4} \pm 7{,}9 \cdot 10^{-5}$ b

ANOVA did not reveal significant differences in the comparison of the progenies from the two treatments with the corresponding parentals (Table 5). In fact, post-hoc tests grouped together the groups within the same treatment (all grafted plants are grouped together and the same goes for the ungrafted plants). Moreover, the values for the ungrafted plants are really close between the progeny and the parentals. In the grafted plants, the progeny has a mean value between both parenrtal, but a higher variability. In general, the variability is higher in the progenies compared to the parentals (there is a ten-fold difference in the standard error).

Table 5. Variability in night-time transpiration (Tr_n ± standard error) among the parentals and progenies from the same treatment (grafted or ungrafted plants). Letters indicate significant differences between different treatments.

	Tr_n (l H_2O day^{-1})
Ungrafted progeny	$4{,}8 \cdot 10^{-2} \pm 1{,}6 \cdot 10^{-2}$ a
Ungrafted Grenache	$5{,}4 \cdot 10^{-2} \pm 9{,}7 \cdot 10^{-3}$ a
Ungrafted Syrah	$4{,}1 \cdot 10^{-2} \pm 6{,}1 \cdot 10^{-3}$ a
Grafted progeny	$1{,}5 \cdot 10^{-2} \pm 6{,}2 \cdot 10^{-3}$ b
Grafted Grenache	$1{,}5 \cdot 10^{-2} \pm 3{,}1 \cdot 10^{-3}$ b
Grafted Syrah	$1{,}5 \cdot 10^{-2} \pm 2{,}3 \cdot 10^{-3}$ b

It was expected not to found significant differences among the progeny and the parents, which already showed a similar behaviour in previous studies. Moreover, genetic variability was also higher in the offspring than in the parents (Coupel-Ledru et al., 2014). It is a trait of interest as the high genetic variability determines differences in the plant behaviour (in parameters like Tr_n or Tr_n/LA) under stress conditions. However, how plants react to stress conditions (like a water deficit) also depends on non-genetic factors, like the evaporative rate, the water stressed regime or the VPD. All of these parameters were set to be as close as possible to the field conditions. So, it is expected than when plants are water-stressed, they may appear more significant differences between the parental genotypes and the progeny, as plants can change their WUE depending on water availability. Another aspect that interferes with the plant parameters studied is the soil volume. "When the roots are kept in a small volume, it minimizes the variability in water relations", said by Pou et al. (2012).

Even the Tr_n/LA parameter was used instead of Tr_n to compare G and UG plants, the older G plants double the values of younger UG plants. There are significant differences in the following genotypes when comparing grafted (G) and ungrafted (UG) plants (Table 4): Grenache, 7G031, 7G036, 7G039, 7G068, 7G092, 7G096, 8S002, 8S012, 8S34, 8S037 and 8S092. A reason to explain the higher differences between treatments could be the accumulated errors from the LA estimates. LA from all 162 was estimated from the veins length, but not all leaves were counted due to technical and logistic difficulties (e.g., the number of plants involved, the number of people working on the experiment, the place where it took place and the lack of time for doing more accurate measurements). However, the error is assumed to be proportional for both groups of plants and that could not explain the significant differences in the results. Therefore, it could be interesting to study those genotypes with lower Tr_n/LA to find more efficient genotypes. It was decided to measure more parameters with the smallest plants, as the effect of the grafting could not be justified by the performed tests. Previous studies showed an effect by the rootstock on the plant mineral nutrition (Lehoczky et al., 1998). The work of Lavoie-Lamoureaux et al. (2016) found significant differences in physiological parameters due to the rootstock used. So, when grafting the same genotype to two different rootstocks, one drought-resistant and one water-sensitive, there are significant differences in stomatal conductance, leaf water potential, A_n and Tr (Lavoie-Lamoureaux et al., 2016; Lovisolo et al., 2016). Even more, the scion's WUE

shows a strong correlation to the rootstock genetic variability. However, both studies focused on the scion-rootstock interaction with water-stressed plants only, unlike the present work.

Genetic variability under different CO_2 concentration conditions

Leaf water potential ranged from -1,4 ± 0,19 to -2,91 ± 1,02 bar (genotypes 8S012 and 8S034, respectively). Considering the osmotic potential, the lowest value was -694,3 ± 83,19 MPa (7G045) and the highest is -558,7 ± 36,86 MPa (8S012) (data not shown). Both leaf water potential and osmotic potentials exhibit common values of non-stressed plants (Fuentes et al., 2014; Rogiers et al., 2011) and there are no significant differences. The goal of these measurements was to make sure all plants were not water-stressed and in the same water status to be able to compare the genotypes in the experiment.

The genotypes showed a wide range of values in the different parameters analysed (day-time transpiration, night-time transpiration, A_n at 1700ppm CO_2 and 400ppm CO_2, growing measurements, leaf water potential and osmotic potential), but ANOVA just revealed significant differences in Tr_d (Table 7). All genotypes had increased A_n at 1700ppm CO_2 compared to 400ppm CO_2 (Table 6). The genotype 8S012 had the greatest increase (from 7,60 ±1,46 to 22,23 ± 2,35 µmol CO_2), but also had the smallest A_n values, while the second highest increase was by genotype 7G046 (from 12,10 ± 4,37 to 33,13 ± 2,80 µmol CO^2), which had the greatest A_n at 1700ppm CO_2. The major A_n at 400ppm CO_2 corresponded to genotype 8S002 (A_n values are 13,13 ± 2,86 and 32,6 ± 2,31 µmol CO_2 at 400ppm and 1700ppm CO_2, respectively).

When considering transpiration measures, there was more variability in Tr_n than in Tr_d. The highest values were by genotype 8S052 (Tr_d) and 7G036 (Tr_n), while the lowest values belonged to genotypes 8S012 (both Tr_d and Tr_n) (Table 7).

Table 6. Variability in the photosynthetic tax (A_n, µmol CO_2 mol^{-1} H_2O ± standard error) at different CO_2 concentrations among the genotypes. Letters indicate significant differences between different genotypes for each parameter separately.

Genotype	**g_s at 400ppm CO_2 (mol CO_2 $µmol^{-1}$ H_2O)**	**g_s at 1700ppm CO_2 (mol CO_2 $µmol^{-1}$ H_2O)**
Grenache	11,133 ± 5,086 a	28,967 ± 9,298 a
Syrah	12,800 ± 5,716 a	31,000 ± 4,866 a
7G036	13,667 ± 4,903 a	27,267 ± 5,036 a
7G045	8,800 ± 4,304 a	22,900 ± 4,900 a
7G046	12,100 ± 4,305 a	33,133 ± 2,801 a
8S002	13,133 ± 2,857 a	32,600 ± 2,307 a
8S012	7,600 ± 1,453 a	22,233 ± 2,350 a
8S034	10,900 ± 1,212 a	28,500 ± 5,724 a
8S040	11,333 ± 2,902 a	26,267 ± 2,444 a
8S052	10,967 ± 4,452 a	24,100 ± 4,386 a

Table 7. Variability in the daytime transpiration (Tr_d ± standard error) among the genotypes. Letters indicate significant differences between different genotypes.

Genotype	**Tr_d (l H_2O day^{-1})**
8S052	0,921 ± 0,056 a
7G046	0,003 ± 0,058 ab
Grenache	0,854 ± 0,023 ab
7G036	0,796 ± 0,085 abc
8S034	0,785 ± 0,044 abcd
8S002	0,751 ± 0,012 abcd
Syrah	0,720 ± 0,068 bcde
8S040	0,657 ± 0,084 cde
7G045	0,620 ± 0,062 de
8S012	0,550 ± 0,064 e

The analysis of the WUE_i (A_n/g_s, both at 400ppm and 1700ppm CO_2) do not show any significant differences between genotypes. The genotype with a higher WUE_i at g_s 400ppm CO_2 is 8S040 (92,802 ± 7,622 mol CO_2 mol^{-1} H_2O), while the less efficient genotype is Syrah (30,587 ± 25,014 mol CO_2 mol^{-1} H_2O) (Figure 5).

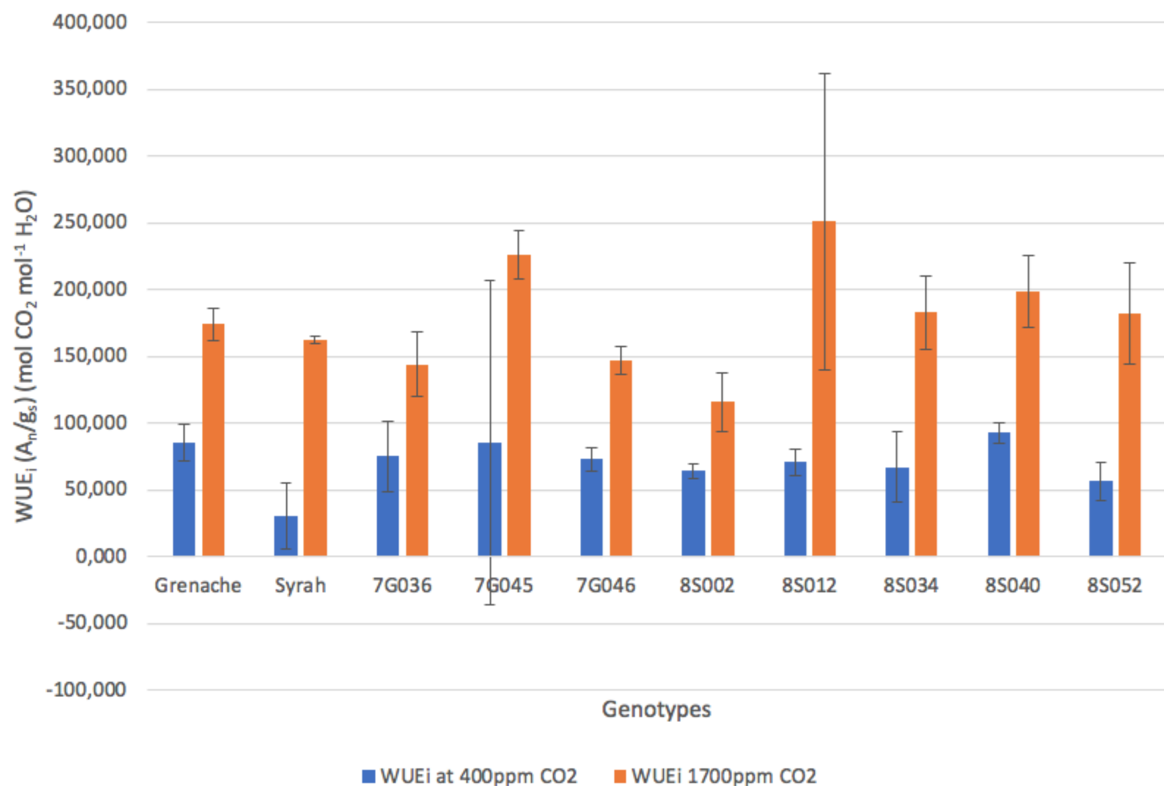

Figure 5. Variability in WUE_i at 400ppm and 1700ppm CO_2 among the genotypes.

Nevertheless, results about the link between transpiration and growth are promising. The ANOVA revealed significant differences in Tr_d, but not in Tr_n, A_n or WUE_i (Table 4, 7, 8, Figure 5). The variation range in A_n is lower than in other genetic variability studies (Bota et al., 2001), but it could be justified by the genotypes origin. In this case, the genotypes come from a progeny with the same parental genotypes, so it was expected less variability than among a collection of different cultivars. Tr_n is a highly variable parameter compared to other studies. That is the reason most studies are based on gas exchange or sap flow measurements (Escalona et al., 2013), because these measures provide Tr estimates.

Genotypes with reduced night-time transpiration and high photosynthetic taxes

To identify the genotypes most efficient genotypes, they were divided by high and low Tr_n and A_n (by using the second quartile), as these are the main target parameters to identify more efficient genotypes in this study (with lower transpiration and higher photosynthetic taxes). 8S002 showed a high A_n (Table 8). Likewise, when genotypes were divided by Tr_n and Tr_d, 8S002 also appeared in the group with lower transpirations, as well as 7G045 and 8S012

(Table 7). Finally, 8S002 was grouped with Syrah as genotypes with high A_n and low Tr_d (Table 11).

When dividing the genotypes by their values in some parameters (Tr_n Tr_d and A_n) (Tables 8, 9 and 10), there's one genotype (8S002) that has low transpiration (both in daytime and night-time) coupled with high A_n. Taking a closer look to its values on these parameters (Table 8), 8S002 experiences the highest growths in a single leaf area and has the lowest Tr_n. In A_n, it has one of the highest values in both CO_2 concentrations. Consequently, 8S002 is a genotype with low transpiration (both during daytime and night-time) and a high photosynthetic tax.

Table 8. Genotypes divided by photosynthetic tax (A_n) and night-time transpirations (Tr_n). They were divided according to the second quartile.

	Low A_n	High A_n
Low Tr_n	Grenache 7G045 8S012 8S052	8S002
High Tr_n	8S034 8S040	Syrah 7G036 7G046

Table 9. Genotypes divided by nighttime and daytime transpiration.

	Low Tr_d	High Tr_d
Low Tr_n	7G045 8S002 8S012	Grenache 8S052
High Tr_n	Syrah 8S040	7G036 7G046 8S034

Table 10. Genotypes divided by daytime transpiration and photosynthetic tax.

	Low A_n	High A_n
Low Tr_d	7G045 8S012 8S040	Syrah 8S002
High Tr_d	Grenache 8S034 8S052	7G036 7G046

The resulting genotype that expresses the desired target may be a promising result, but it represents a starting point for further studies that support the conclusions obtain in the present one. In the future, sampling methods experiment conditions should be improved to achieve more relevant results. However, previous studies with similar conditions (Coupel-Ledru et al., 2014) revealed that a reduction in transpiration may be also determined by hydraulic conductance and stomatal control, not only be the water availability in soil. Due to the nature of this study, it is key to analyse a wide range of genotypes in order to find some that express the target parameter (reduced night-time transpiration, in this case). On one hand, the culture chamber has a limited space to contain plants and on the other hand, the greenhouse can

contain a lot more of them, but needs more people to manage the experiment due to the plant number. Then, it could be interesting a sampling method that allows collecting many data at the same time, that it is easily preserved and can be comfortably analyzed at the laboratory, like the δ^{13} in berries (Bchir et al., 2016). δ^{13} in berries gives an estimate of the WUE of plants during the berry formation (according to the plant cycle, it corresponds to the summer period, when there is less water availability in the Mediterranean climate), thus providing data for the WUE of the plant during a period of time. Other studies focus on ancient cultivars not so currently used (Bota et al., 2015) as a source for more resistant plants. Nonetheless, Bota et al. (2015) did not find a genotype with all the traits of interest, but concluded that those cultivars represent a gene pool useful for further experiments.

Conclusions

In summary, the tests support there is an interaction scion-rootstock that should be further analysed. This interaction interferes with plant transpiration and could help to obtain more water efficient genotypes and a more sustainable agriculture. Indeed, there is a promising genotypes (8S002) with reduced Tr_n and increased A_n resulting from the Syrah x Grenache progeny, which exhibits the characters of interest (high photosynthetic tax with low transpiration). It could be explained by the plants water regimes, as water deficit triggers water-saving strategies in plants and ours were well watered during all the experiment.

Further studies should be performed in phenotyping platforms to avoid technical errors and considering other target parameters (like δ^{13} in berries). They also should feature contrasting water regimes and rootstock to go in deep in the nature of scion-rootstock interactions and water-saving plant strategies.

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