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Ecophysiological responses of grapevine rootstocks to water deficit

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Summary

The use of rootstocks tolerant to soil water deficit is an interesting strategy to face the challenges posed by limited water availability. Currently, several nurseries are breeding new genotypes aiming to improve the water stress tolerance of grapevine, but the physiological basis of its responses under water stress are largely unknown. For this purpose, an ecophysiological assessment of the conventional 110-Richter (110R) and SO4, and the new M1 and M4 rootstocks was carried out in ungrafted potted plants. During one season, these *Vitis* genotypes were grown under greenhouse conditions and subjected to two water regimes, well-watered (WW) and deficit irrigation (DI). Water potentials of plants under DI down to <-1.4 MPa, and net photosynthesis (A_N) <5 μmol CO_2 m^{-2}s^{-1} did not cause leaf oxidative stress damage compared to WW conditions in all genotypes. The antioxidant capacity was sufficient to neutralize the mild oxidative stress suffered. Under both water regimes, gravimetric differences in daily water use were observed among genotypes, leading to differences in the biomass of roots and shoots. Under WW conditions, SO4 and 110R were the most vigorous and M1 and M4 the least. However, under DI, SO4 exhibited the greatest reduction in biomass, while 110R showed the lowest. Remarkably, under these conditions, SO4 reached the least negative stem water potential and showed the highest hydraulic conductance values. Conversely, M1 reduced the most stomatal conductance, transpiration and A_N. Overall, 110R achieved the highest biomass water use efficiency in response to DI, and SO4 the lowest, while M-rootstocks showed intermediate values. Our results suggest that there are differences in water use regulation among genotypes attributed not only to differences in stomatal regulation but also to plant hydraulic conductance. Therefore, it is hypothesized that differences in genotype performance may be due to root anatomical-morphological differences and to several physiological processes such as growth inhibition, osmotic adjustment, antioxidant production, nutrient translocation capacity, etc. Further studies are needed to confirm these differential ecophysiological responses of *Vitis* species under water stress, particularly under field and grafted conditions.

Keywords

antioxidant metabolism, biomass, chlorophyll fluorescence, leaf gas exchange, hydraulic conductance, water use efficiency

Introduction

Traditionally, grapevine has been considered a dry-land crop because of its capacity for adapting to limited water conditions (Medrano *et al.*, 2015). Thus, grapevine is a crop with a great tradition that occupies quite extensive areas in semi-arid regions (Cifre *et al.*, 2005; Pou *et al.*, 2008). However, the increase of temperature and change of rainfall patterns due to climatic change conditions (IPCC, 2021) make grapevines highly vulnerable to environmental changes (Schultz, 2017; Sadras *et al.*, 2017). In addition, factors such as demand from winegrowers to stabilize yields while improving grape quality under changing climate conditions, make irrigation a more regular practice in viticulture (Chaves *et al.*, 2007; Intrigliolo and Castel, 2010; Bonada *et al.*, 2018). It is known that irrigation increases photosynthesis, vegetative and reproductive growth and yield by 1.5 to 4 fold, although this response depends on several factors such as irrigation timing, amount of water applied, cultivar and rootstock, environmental conditions and other cultural practices (Escalona *et al.*, 2003; Cifre *et al.*, 2005; Bascuñán-Godoy *et al.*, 2017). However, high amounts of irrigation also can provoke imbalanced grapevines affecting grape quality and furthermore maybe not sustainable under limited water conditions (Medrano *et al.*, 2015; Romero *et al.*, 2019). Thus, the use of rootstocks for

improving drought tolerance and water use efficiency seems to be a promising strategy to meet the challenges of climate change (Alsina *et al.*, 2011; Ollat *et al.*, 2016; van Leeuwen and Destrac-Irvine 2017; Romero *et al.*, 2018; Marín *et al.*, 2021). Nevertheless, while field practices have been largely studied, the role of the genetic material and, particularly, the rootstock, in improving vine performance under water stress requires further attention (Zhang *et al.*, 2016).

Traditionally, grafting has been used in European *Vitis vinifera* L. varieties to combat pests such as Phylloxera (*Daktulosphaira vitifoliae* Fitch), but also to adapt vineyards to soil conditions (Quiroga *et al.*, 2017; van Leeuwen *et al.*, 2018). Nowadays, it is known that rootstocks have a direct impact on vine development, both vegetative and reproductive growth, biomass accumulation, vine phenology and grape ripening (Ollat *et al.*, 2016; Bordenave *et al.*, 2018; Kodur *et al.* 2013). Rootstocks also induce tolerance to biotic and abiotic limiting factors such as salinity, drought, or nutritional deficiencies (Keller *et al.*, 2001; Marguerit *et al.*, 2012; Serra *et al.*, 2014; Zamboni *et al.*, 2016; Bianchi *et al.*, 2018, 2020a). Rootstocks can influence stomatal conductance, transpiration and leaf photosynthesis and thus vine vigor (Gambetta *et al.*, 2012; Lavoie-Lamoureux *et al.*, 2017), leaf and bunch mineral composition (Zamboni *et al.*, 2016; Bianchi *et al.*, 2020b), and yield and grape composition (Romero *et al.*, 2018; 2019). However, currently, and according to Serra *et al.* (2014), only 10 rootstocks are used for grafting for about 90% of grapevine genotypes established around the world. Although there is a certain degree of variability within each rootstock genotype (Peiró *et al.*, 2020), this genetic background is very limited, which is an important constraint for choosing the optimal rootstock for each variety and climatic scenario (Riaz *et al.*, 2019). Therefore, breeding programs of new rootstock genotypes, with more diverse and better performances, are needed for improving sustainability of the grape industry (Serra *et al.*, 2014; Bordenave *et al.*, 2018).

Thus, research groups such as that of the Department of Agricultural and Environmental Sciences (DiSAA) of the University of Milan (Italy), worked to obtain new rootstocks able to face abiotic stresses (Bianchi *et al.*, 2018). As a result of these works, breeding genotypes named "M-series" have been achieved, which are tolerant to drought, active lime and salinity as well as they have the ability to differentially control vine vigor (Porro *et al.*, 2013; Zamboni *et al.*, 2016). The M1, M2, M3 and M4 rootstocks have different characteristics that now should be evaluated at the physiological and agronomic levels. Among these rootstocks, it has been observed that M4 rootstock increases drought tolerance due to better conservation of the integrity and functionality of the root-tissue conferring high water use efficiency to the scion (Porro *et al.*, 2013; Meggio *et al.*, 2014; Merli *et al.*, 2014; Corso *et al.*, 2015; Galbignani *et al.*, 2016; Frioni *et al.*, 2020). Corso *et al.* (2015) reported the activation of detoxification or reactive oxygen species in the M4 rootstock genotype compared to 101-14 rootstock. However, scarce literature provides few information comparing the M-series without grafting with other widely used rootstocks and, therefore, no conclusions can be drawn about the response to abiotic stresses independently of the interactive effect the of scion (Clingeleffer *et al.*, 2019).

Among traditional rootstocks, Selection Oppenheim nº 4 (SO4) and Richter-110, (110R) are among the more widely used in viticulture. In general, SO4 has been classified as medium-high vigor rootstock, resistant to Phylloxera, and lowly resistant to Petri disease, Crown Gall, and medium resistant to nematodes (Schmid *et al.*, 1998; Ferreira *et al.*, 2018). Besides, SO4 has medium resistance to iron chlorosis and to low temperature (Bavaresco and Lovisolo, 2000). SO4 has been classified among the less drought tolerant rootstocks (Marín *et al.*, 2021). However, the hybrid 110R has been classified as highly drought tolerant (Ollat *et al.*, 2016; Pou *et al.*, 2008). According to Galmés *et al.* (2007), under different intensities of water stress, 110R confers to scion an isohydric behavior. This means that by strict regulating of the stomatal conductance by abscisic acid (ABA) control, 110R rootstock is able to avoid dehydration, maintaining homeostasis in its leaf water relations (Pou *et al.*, 2008). Moreover, Boso *et al.* (2016) reported differences in stomatal density between SO4 and 110R. This is hypothesized to underlie different strategies in stomatal regulation. In spite of these works, a better understanding of the physiology of drought stress in *Vitis* sp. is needed to face the challenges posed by climate change (Zhang *et al.*, 2016; Shtein et al., 2017).

In the present research, the morphological and physiological responses to water stress of two novel genotypes, M1 and M4, were compared to two traditional rootstocks widely used in viticulture, 110R and SO4. The objective was to know their relative tolerance to the drought condition and, identify the mechanism involved in water stress responses. For this, ungrafted plants in 25 L pots were grown under greenhouse conditions under two different water regimes: Deficit irrigation (DI) and well-watered (WW). Vine performance and physiological responses of the plants, including biomass allocation, stem water potential, leaf gas exchange parameters, hydraulic conductance, leaf chlorophyll content and fluorescence, and oxidative activity were measured.

Material and Methods

1. Plant material and growing conditions

Ungrafted two year-old grapevine plants (*Vitis* sp.) were transplanted into pots after trimming the root tips. The pots were 25 L in volume and were filled with a mixture of organic substrate (blond peat) and perlite (3:1). The assay was conducted in a multi-tunnel greenhouse at the CEBAS experimental field (Santomera, Murcia, Spain) during the 2018 season. To control temperature, a cooling system and aluminum shading nets were used to intercept 30% of the incident radiation. The environmental conditions inside the greenhouse during the study were: day/night temperature 30/18 ± 3◦C, relative humidity 54/82 ± 5%, and a photoperiod of 15 h with average photosynthetically active radiation of ~1,100 µmol m^{-2} s^{-1} at solar noon.

The experimental design consisted in 4 different rootstock genotypes (G) subjected to 2 different water regimes (WR):

well-watered (WW) and deficit irrigation conditions (DI). Each combination rootstock × water regime had a total of 12 plants, for a total of 96 experimental plants, randomly placed in the greenhouse in 3 blocks with four plants (repetitions) per block. The rootstocks used were: Richter-110 (*V. berlandieri* cv. Boutin × *V. rupestris cv. Du Lot*), and SO4 (*V. berlandieri* cv. Resseguier nr.2 × *V. riparia* cv. Gloire de Montpellier), widely used in world viticulture and, two recently bred ones: M1 [(106-8 (*V. riparia* × (*V. cordifolia* × *V. rupestris*)) × *Resseguier* n.4 (*V. berlandieri cv. Planchon*))], and M4 [[(*V. vinifera* × *V. berlandieri*) × *V. berlandieri* cv. Resseguier no. 1].

Well-watered plants were irrigated to field capacity throughout the experiment (15 minutes of irrigation, twice a day). Deficit irrigation plants were watered daily at 35% of the reference evapotranspiration (ET$_o$), with a daily irrigation of 4-6 minutes. Each plant was provided with two irrigation micro-tubes with drippers of 1 L h^{-1}. All plants received Hoagland nutrient solution at 50%, applied during irrigation in the whole experiment. This nutrient solution contains: KNO$_3$ (27 g 100 L^{-1}), Ca(NO$_3$)$_2$ (42 g 100 L^{-1}), KH$_2$PO$_4$ (7 g 100 L^{-1}), MgSO$_4$ (13 g 100 L^{-1}), Fe-EDTA (1 g 100 L^{-1}), as well as a commercial mixture of micronutrients (1 g 100 L^{-1}; (Hidromix S, Valagro, Chieti, Italy)).

Two shoots per vine were allowed to grow from two 1-node spurs kept at the beginning of the assay. Shoot growth was directed upward along the catch wires to fill all the available space and gain the best foliage light exposure while minimizing mutual shading. Shoots were trimmed when they reached ~2.1 m in height. In addition, secondary shoots were removed twice during the season. The experiment was carried out from August to October 2018, meanwhile, the correspondent agronomic and physiological measurements were performed.

2. Water use, soil and plant water status

Vine water use was estimated by daily water balance. It was determined by subtracting the weight at 5.00 h am (solar time) and on the consecutive day (24 hours). No irrigation was applied during the 24 hours when pot weight determinations were conducted. The weighing of two pots of each G × WR combination per block (n = 6) were carried out weekly from September to October (6 times/season). Water use over the experiment was calculated as the average daily use multiplied by the number of days from the 5th September to 19th October (WU, L/plant).

In order to estimate grapevine water status, midday stem water potential (Ψ_{stem}) was measured with a Scholander pressure chamber (Soil Moisture Equipment Corp., Sta Barbara, CA, USA). Leaves were covered with an aluminum foil bag for at least 45 min before measurement and subsequently, the base of the petiole was excised with a sharp razor and the leaf was inserted immediately into the chamber. Ψ_{stem} was measured weekly from September to October on one leaf per plant in two plants for combination G × WR per block (n = 6).

The soil water characteristic curve, which relates soil water potential (Ψ_{soil}) to water content, was calculated by a pressure plate extractor following Dane and Hopmans (2002) procedure. Briefly, 10 aluminum cylinders 37 mm in diameter and 15 mm high filled with the experimental substrate were placed on porous ceramic plates with an impermeable bottom and drainage system and were subjected to different pressures (0.1, 10, 33, 80, 400, 800 and 1,500 kPa). At each pressure point, the wet and dry weights of each cylinder were determined in order to calculate the gravimetric moisture content. For each measuring date, the water content was estimated as the total pot weight minus the weight of the container, the fresh weight of the plant and the weight of the substrate at wilting point (1,500 kPa), estimated from the field capacity weight of the first weighing date. Finally, the soil water characteristic curve was used to calculate Ψ_{soil} at each weighing date. These data were used to calculate hydraulic conductance (see "4. Root-to-stem hydraulic conductance" section).

3. Leaf gas exchange

Leaf net photosynthesis (A$_N$; μmol CO$_2$ m^{-2} s^{-1}), stomatal conductance (g$_s$; mol H$_2$O m^{-2} s^{-1}) and transpiration rate (E; mmol H$_2$O m^{-2} s^{-1}) were measured with a portable infrared gas analyzer LCPro + Portable (ADC, Bioscientific, Ltd, UK). Gas exchange determinations were measured midday between 10.00 and 12.00 h solar time in the same days of Ψ_{stem}. One mature and healthy leaf per plant was selected on two plants of each combination G × WR per block (n = 6). The ratio of A$_N$ and g$_s$ was used for calculating the intrinsic water use efficiency (WUE$_i$; μmol CO$_2$ mol^{-1} H$_2$O).

4. Root-to-stem hydraulic conductance

Hydraulic conductance (K$_h$) from root to stem was estimated using the evaporative flux method (Nardini and Salleo, 2000), which is based on an Ohm's law hydraulic analog, using the following equation (Tsuda and Tyree, 2000):

$$K_h = E/(\Psi_{soil} - \Psi_{stem})$$

where K$_h$ is the root to stem hydraulic conductance, E is the maximum diurnal transpiration, while $\Psi_{soil} - \Psi_{stem}$ represents the maximum diurnal drop in water potential of the soil-plant-atmosphere continuum. Two approaches were used to estimate E. The first approach used the daily water use by the entire plant calculated by water balance (K$_h$ WB). The second approach consisted in scaling E assessed by gas exchange (K$_h$ GE) at leaf level to the total leaf area of the vine at each measuring date. It should be noted that the daily water use calculated by water balance takes into account both, the evaporation of the substrate and vine transpiration.

5. Leaf chlorophyll content

Fully developed basal, medium and apical leaves in all orientations were used to measure chlorophyll content index (CCI) with a portable Cl-01 chlorophyll content meter (Hansatech Instruments Ltd., Norfolk, UK). This device determines the relative content of chlorophyll using dual-wavelength optical absorbance (620 and 940 nm) from sample leaves. For these measurements, two healthy leaves per every type in 6 plants of G × WR combinations were used. The days on which the measurements were taken coincided with the days on which

the water potential and gas exchange determinations were made.

6. Chlorophyll fluorescence

Chlorophyll fluorescence measurements were performed on the same days and the same leaves used for measuring the gas-exchange parameters, using a modulated pulse portable fluorimeter (*FMS-2*, Hansatech Instruments). This equipment was used to do the fluorescence kinetics of leaves adapted to light (Olmo *et al.*, 2019). The parameters measured were: PSII quantum yield, $\Phi PSII = Fv'/Fm' \times qP$; antennae efficiency of PSII, $Fv'/Fm' = (Fm' - F0')/Fm'$; and photochemical quenching decay, $qP = (Fm' - Fs)/(Fm' - F0')$, where Fs is the fluorescence performance in a stable state, Fm' is the maximum value when all the reaction centers are closed after a saturating light pulse [12,000 $\mu mol(photon)$ m^{-2} s^{-1} for 0.8 s], and F0' is the minimum fluorescence in the state of adaptation to the light obtained by temporarily turning off the actinic light and applying a pulse of far-red light (735 nm) to drain the PSII of electrons. For these fluorescence measurements, two healthy and mature leaves in 6 plants of G × WR combinations were used. The days on which the measurements were taken coincided with the days on which the water potential and gas exchange determinations were made.

7. Oxidative damage

At the end of the experiment, before complete harvest of the plants, two mature and healthy leaves per plant (six plants per each genotype and water regime combination) were frozen in liquid nitrogen for the oxidative stress study. The quantification of H_2O_2 was performed following the method described by Yang *et al.* (2007). Lipid peroxidation was determined by measuring malondialdehyde (MDA) using the method of Hodges *et al.* (1999). For the determination of antioxidant activity, briefly, 250 mg of frozen material were utilized for the extraction, with this tissue homogenized in 2.5 mL ethanol (80%). Posteriorly, the samples were centrifuged at 10,000 rpm for 15 min and the supernatant was utilized to measure antioxidant activity with the DPPH method described by Koleva *et al.* (2002).

8. Vegetative growth: leaf area, shoots and roots length and total biomass

During the trial, the green prunings were carried out on 22th August and 14th September. The secondary and cut shoots were measured and weighed and the leaf area of the removed leaves was estimated.

Before each physiological measurement date, two medium-size secondary shoots per plant were measured on each experimental plant to calculate its growth rate and estimate total vine leaf area. At this time, secondary shoots were counted on each plant. Total leaf area per vine (LA) was estimated using linear equations relating LA to shoot length (main and secondary shoots), following the procedure described by Buesa *et al.* (2019). Briefly, this relationship was established by determining LA (LI-3100 Area Meter, LI-COR Inc., Lincoln, NE, USA) and total shoot length in 10 shoots of different lengths, separating main and lateral shoots.

In addition, at the end of the trial (19th October), 48 plants (2 plants of each G × WR combination; n = 6) were used for measuring length of the two main shoots of each plant and its secondary shoots, cutting and weighing them (fresh weight). Also, the whole root systems of these plants were taken out of the pots, cleaned with tap water, dried in the sun, weighed and the length of the main root was measured. Photographs were taken of each of the cleaned roots. Both, the aerial part and the roots per plant were placed in an oven until constant weight (70 °C) to obtain dry weight. Using these, the shoot-to-root ratio was calculated as: (dry mass of leaf + stem)/dry mass of root.

From the sum of both, the fresh weights of the biomass obtained in the trimming and the fresh weight of the main and secondary shoots at the end of the season, the total weight of fresh aerial matter was obtained. Likewise, the sum of the corresponding weights after drying made it possible to calculate the dry weight of aerial matter. By ratio of dry to fresh matter, it was possible to calculate the water content of the aerial part (WC) of the plants under each of the water regimes.

Moreover, water use efficiency in terms of total biomass (WUE_b) was estimated as the ratio between total fresh biomass and the amount of total water used per plant over the experiment (WU).

9. Statistical analysis

The evaluation of the effects of the factors and their interactions on the studied variables was carried out by means of a three-way analysis of variance (ANOVA) when determinations were repeated over the season, and by a two-way ANOVA for parameters assessed only at the end of the experiment. All the factors evaluated (genotype, G; water regime, WR; and date, D) had significant effects on most of the variables. As significant interactions between G × WR were detected for many of the variables considered (Supplementary Tables 1 and 2), data are shown in average of G × WR combination. Mean separation was carried out via Duncan´s test (significant at $p < 0.05$). In addition, in an attempt to explain the common behavior of the genotypes, the relationship between all the variables studied throughout the experiment was assessed by a matrix correlation. Statistics were performed using the "Statgraphics Centurion XVI" package version 16.0.07 (StatPoint Technologies, Inc., Warrenton, VA, USA) and regressions were obtained using SigmaPlot (version 11.0) (Systat Software).

Results

The first analysis showed that both genotype and water regime significantly affected most of the parameters evaluated. In addition, it showed that the triple (GxWRxD) and double (GxD) interactions were not significant for any parameter (Supplementary Tables 1, ST1). The WRxD and GxWR interactions, however, were significant for some parameters. The

former is attributable to the fact that the soil water status during the experiment varied among dates to a greater extent in the DI treatment than in the WW treatment. On the other hand, the GxWR interaction points to interesting differences in genotype responses as a function of the water regime. Based on this, most of the parameters are presented as the average of the dates for each genotype and water regime combinations.

The second analysis showed that genotype, water regime and their interaction were significant for most of the parameters evaluated at the end of the experiment (Supplementary Tables 2, ST2). Therefore, these parameters are presented as the mean per water regime and genotype combination.

1. Seasonal evolution of ecophysiological traits

1.1. Water status relations and gas exchange parameters

Overall, the Ψ_{stem} showed a significant effect of WR and D, but not of G (ST1), there was, however, a significant GxWR interaction. In fact, pooling data across the experimental period, there were no statistically significant differences among genotypes in vine water status under WW, but under DI (Table 1). Under WW conditions, vines maintained, during most part of the experiment, Ψ_{stem} values around -0.6 to -0.8 MPa (Fig. 1). This vine water status corresponded to leaf net assimilation rates (A_N) ranging from 10-12 μmol m^{-2} s^{-1} and stomatal conductance (g_s) around 0.20 to 0.25 mol m^{-2} s^{-1} (Fig. 1), without showing significant differences among genotypes (Table 1).

When DI was applied, Ψ_{stem}, g_s A_N and E were clearly reduced compared to WW conditions (Table 1) and the lowest values were recorded in all genotypes in the determinations carried out on September 26 and October 17 (Figure 2). Pooling data across the experimental period, the SO4 vines showed significantly less negative Ψ_{stem} values, about 0.20 MPa higher than the 110R and M1 genotypes (Table 1), not differing from M4. In relation to gas exchange parameters under DI, the M1 vines stand out by lower A_N, g_s, and E values than the other genotypes (Fig. 2; Table 1).

Differences in WUE_i among rootstock genotypes were not found in both water regimes (Table 1). All genotypes significantly increased WUE_i by approximately 50% under DI conditions compared to WW. Since part of the variability in WUE_i is explained by changes in g_s, and in our trial there were differences among genotypes in g_s under DI conditions (Table 1), the relationship between WUE_i and g_s was evaluated for each genotype (Fig. 3a). A comparison of individual relationships confirmed that the response of WUE_i against g_s was similar among genotypes, thus only the general regression is shown.

In order to assess differences in stomatal regulation among genotypes, the relationships between g_s and Ψ_{stem} were assessed (Bota *et al.*, 2016). This analysis showed no differences in the slope of these regressions and therefore only the general relationship between g_s and Ψ_{stem} is shown (Fig. 3b). Moreover, no differences were neither detected in the ratio of internal to atmospheric CO_2 concentration (c_i/c_a) between WR (0.66 and 0.65 in WW and DI, respectively), nor among genotypes (data not shown).

Differences in WU among rootstock genotypes were consistently found under both water regimes (ST1). As expected, all genotypes significantly decreased WU under DI conditions compared to WW (Table 1). Under WW conditions, the 110R and the SO4 showed higher WU compared to M4, but not to M1. The highest decrease from WW to DI was observed in 110R and M1, with a reduction about 59.0% and 52.1%, respectively. Consequently, under DI conditions, these two genotypes significantly used less water than M4 and SO4.

1.2. Hydraulic conductance

Root to stem hydraulic conductance was calculated by two methodological approaches, K_h (WB) and K_h (GE). Under both approaches, this parameter was significantly affected by G, WR, D and WRxD (ST1). Under both WR, the SO4 genotype showed the highest values in K_h (WB). Whereas, in K_h (GE) there were only significant differences among genotypes under DI conditions (Table 2). Overall, K_h (WB) decreased in all four genotypes by 76% on average compared to WW conditions (Table 2). In K_h (GE), however, there was an interactive

Table 1: Water relations variables and gas exchange parameters across the experimental period (stem water potential (Ψstem); leaf photosynthesis rate (AN); stomatal conductance (gs); leaf transpiration (E); Intrinsic water use efficiency (WUEi) and Water use (WU)) in four rootstock genotypes (110R, M1, M4 and SO4) of well-watered (WW) and deficit irrigated (DI) plants.

Parameter	Ψ_{stem} (MPa)		A_N (μmol CO_2 m^{-2} s^{-1})		g_s (mol H_2O m^{-2} s^{-1})		E (mmol H_2O m^{-2} s^{-1})		WUE_i (μmol CO_2 mol^{-1} H_2O)		WU (L plant^{-1})	
Factor	WW	DI	WW	DI	WW	DI	WW	DI	WW	DI	WW	DI
110R	-0.67	-1.27a*	10.3	7.6b*	0.224	0.123b*	2.9	1.9ab*	48.8	75.3*	44.4b	18.2a*
M1	-0.64	-1.26a*	8.7	4.7a*	0.210	0.078a*	2.8	1.5a*	43.4	69.4*	38.8ab	18.6a*
M4	-0.65	-1.19ab*	8.9	7.3b*	0.201	0.120b*	2.8	2.0b*	46.8	71.9*	33.1a	20.5b*
SO4	-0.69	-1.06b*	9.1	6.8b*	0.226	0.152b*	3.2	2.3b*	45.1	58.0*	44.6b	26.1b*

Data are averages of 6 dates during the 2018 season for each genotype and water regime combination. WW, Well-watered; DI, Deficit irrigation; Within each parameter and water regime, mean values followed by a different letter are significantly different at P<0.05; * means differences between irrigation treatments for that parameter and genotype.
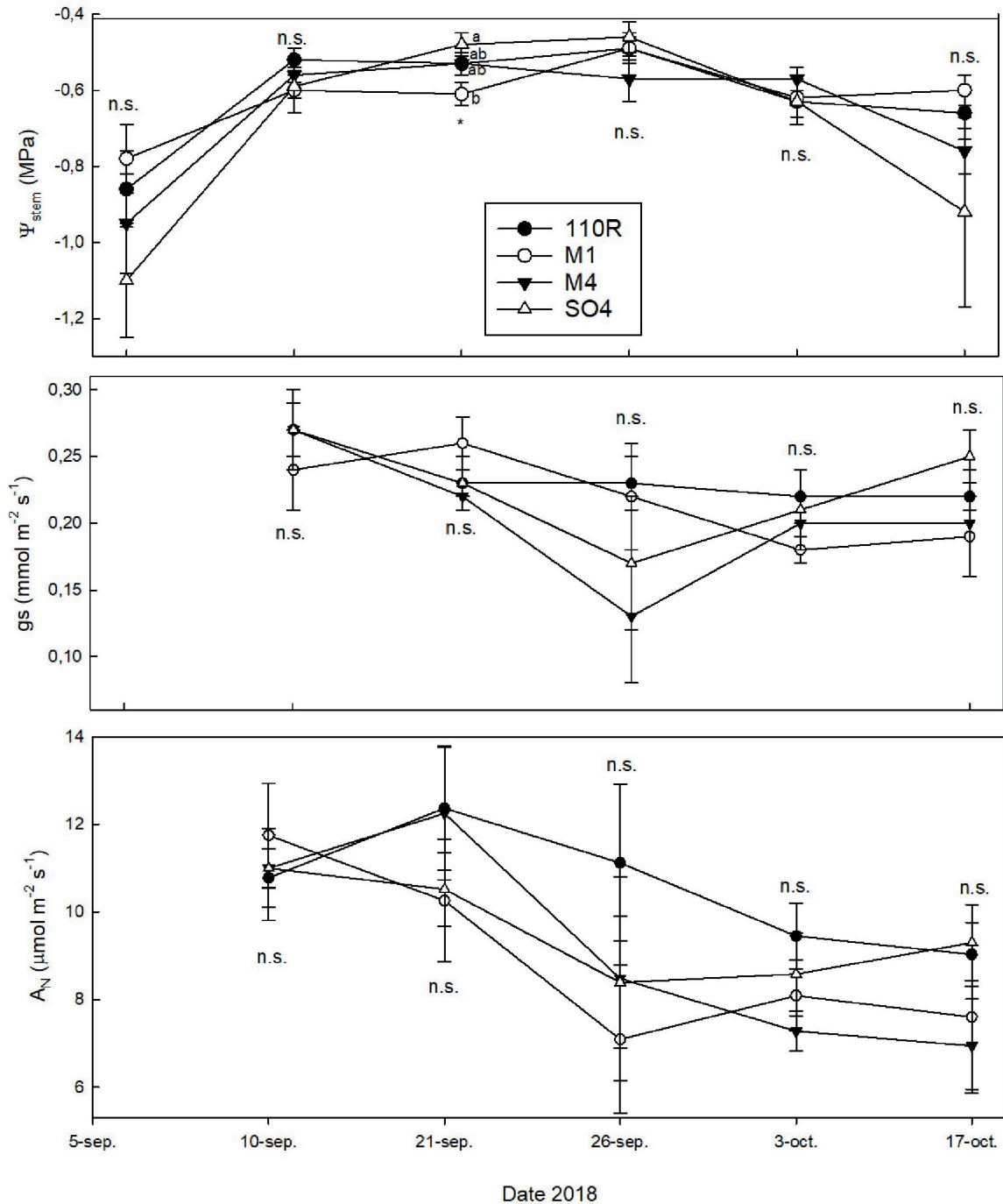
Fig. 1: Seasonal evolution of midday stem water potential (Ψ_{stem}), leaf stomatal conductance (g_s) and leaf photosynthesis rate (A_N) in Santomera, Murcia, Spain. Data are average and standard error values of six determinations per combination of genotype under well-watered conditions (WW).

effect GxWR because SO4 did not reduce its values between WR in contrast to what was observed in 110R, M1 and M4 (ST1). In fact, under DI conditions, SO4 showed no reductions in K_h (GE) between WW and DI, while 110R, M1 and M4 showed significant reductions by 29, 48 and 33%, respectively (Table 2).

1.3. Chlorophyll content and fluorescence

Chlorophyll content was significantly affected by G and D in both basal and medium-apical leaves (ST1). WR had a signif-

icant effect on basal chlorophyll content and GxWR on medium-apical leaves. This implies that water stress affected $Chl_{mid-apic}$ in a genotype-dependent manner (Table 3). Under DI conditions, the leaf $Chl_{mid-apic}$ concentration was not affected in 110R, M1 and M4, but it was significantly reduced by 15.62% in SO4. Overall, 110R and M1 genotypes showed the highest values of this parameter.

Leaf Chl_{basal} was higher under DI than WW in all genotypes, being 61%, 54%, 65%, and 26% higher for 110R, M1 and M4 than for SO4, respectively, compared to its respective WW ones. Thus, under DI conditions, the highest leaf Chl_{basal} was
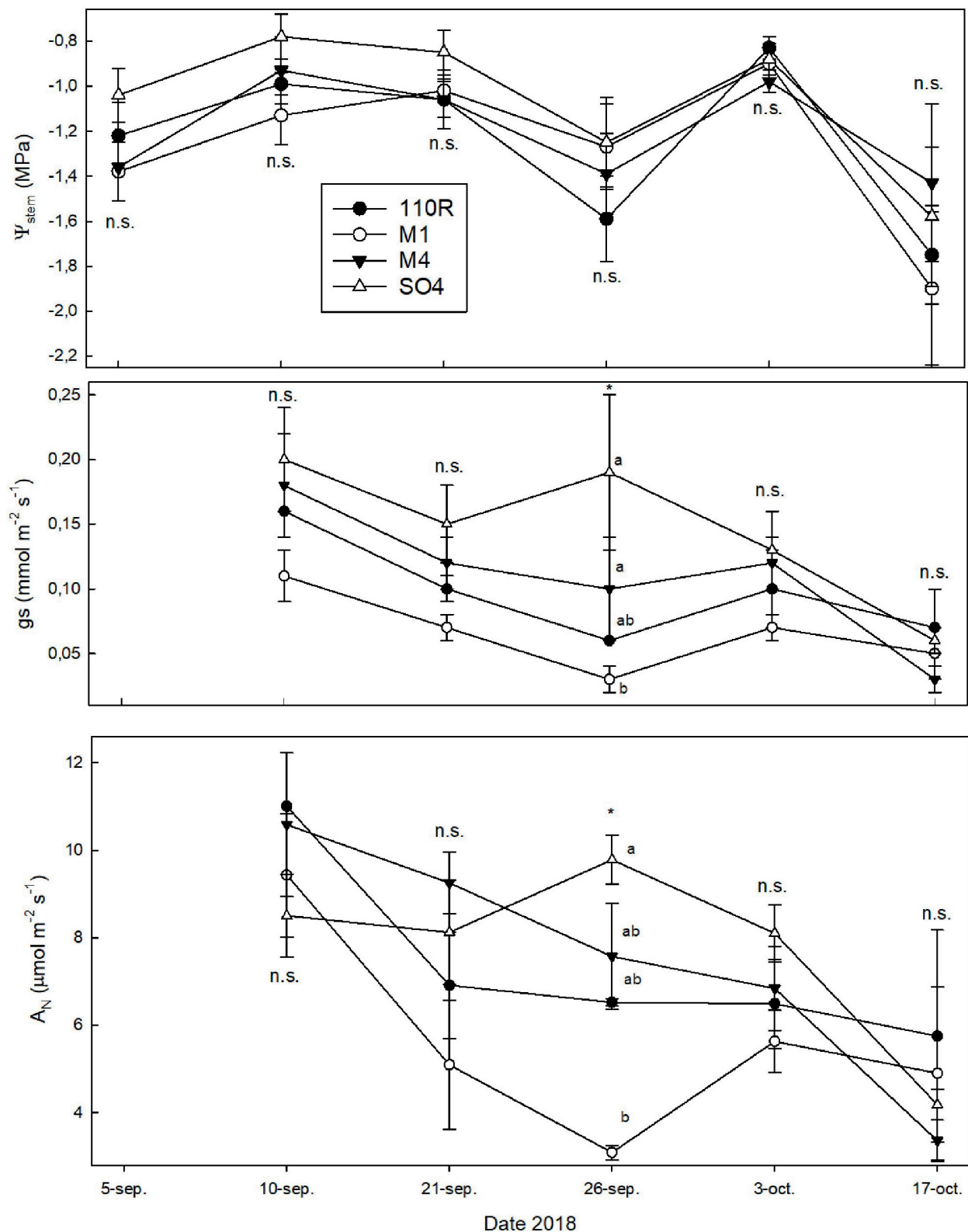
Fig. 2: Seasonal evolution of midday stem water potential (Ψ_{stem}), leaf stomatal conductance (g_s) and leaf photosynthesis rate (A_N) in Santomera, Murcia, Spain. Data are average and standard error values of six determinations per combination of genotype under deficit irrigation conditions (DI).

found again in 110R and M1 genotypes, whereas, under WW, Chl_{basal} did not differ among genotypes (Table 3).

Regarding chlorophyll fluorescence, only the D factor significantly affect these parameters (ST1). A reduction in chlo-

rophyll fluorescence parameters was observed on the last measurement date (data not shown). This means that there were no noticeable changes in the photosystem II activity and chlorophyll fluorescence traits under water deficit conditions, nor in relation to the genotype used.
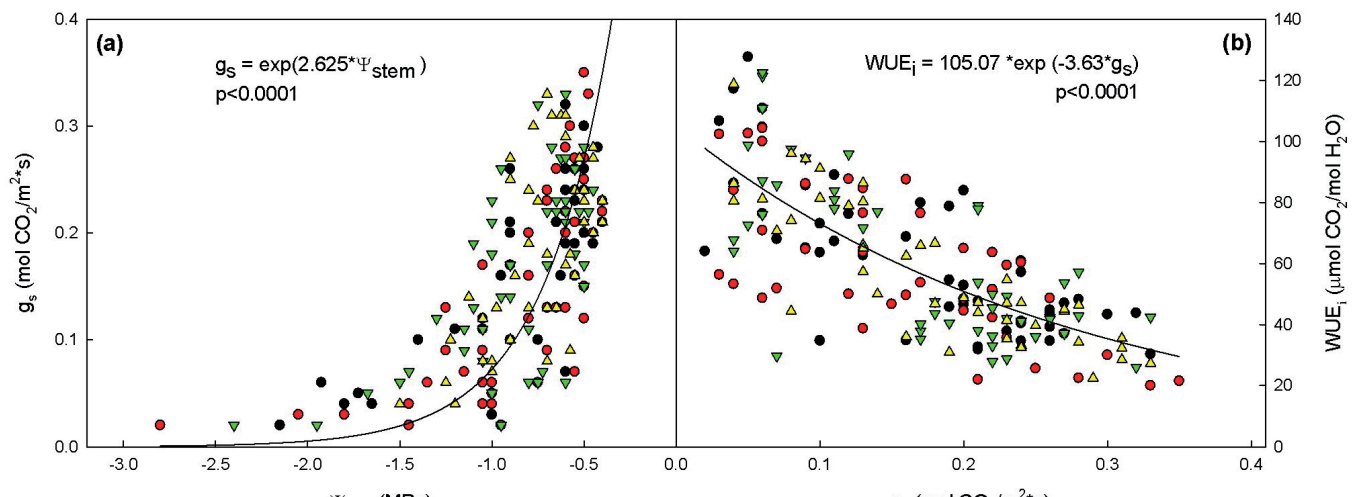
Fig. 3: Relationship between (a) leaf stomatal conductance (g_s) and stem water potential (Ψ_{stem}) and (b) intrinsic water use efficiency (WUE_i) and stomatal conductance (g_s) of 4 rootstock genotypes (●, 110R; ●, M1; ▼, M4; ▲, SO4) at 6 dates of 2018 in Santomera, Murcia, Spain. Fitted regression for all rootstocks is shown.

Table 2. Root to stem hydraulic conductance (K_h) estimated either by water balance (WB) or by gas exchange (GE) across the experimental period in four rootstock genotypes (110R, M1, M4 and SO4) of well-watered (WW) and deficit irrigated (DI) plants.

Parameter	K_h WB (mmol H_2O MPa^{-1} m^{-2} s^{-1})		K_h GE (mmol H_2O MPa^{-1} m^{-2} s^{-1})		K_h reduction (%)	
Factor	WW	DI	WW	DI	WB	GE
110R	8.5ab	1.6ab*	7.4	4.5b*	80	29ab
M1	7.6ab	1.3a*	5.7	2.6a*	82	48b
M4	6.7a	2.3bc*	7.1	4.4b*	76	33b
SO4	10.3b	2.5c*	7.3	5.9b	75	15a

Data are averages of 6 dates during 2018 season for each genotype and water regime combination. WW, Well-watered; DI, Deficit irrigation; K_h WB, hydraulic conductance assessed by water balance; K_h GE, hydraulic conductance assessed by gas exchange. Within each parameter and water regime, mean values followed by a different letter are significantly different at P<0.05; * means differences between water regimes, for that parameter and genotype.

Table 3. Chlorophyll content index (CCI) in apical shoots (apical) and fully expanded leaves (medium), and the chlorophyll fluorescence parameters (chlorophyll content in basal leaves (Chl_{basal}); chlorophyll content in medium and apical leaves ($Chl_{mid-apic}$); performance of the PSII antenna (Fv'/Fm'); quantum efficiency of PSII (ΦPSII); photochemical coefficient (qP)) in four rootstock genotypes (110R, M1, M4 and SO4) of well-watered (WW) and deficit irrigated (DI) plants.

Parameter	Chl_{basal}		$Chl_{mid-apic}$		ΦPSII		Fv'/Fm'		qP	
Factor	WW	DI	WW	DI	WW	DI	WW	DI	WW	DI
110R	4.6	7.4b*	7.9c	7.5b	0.64	0.60	0.73	0.69	0.87	0.86
M1	4.6	7.1b*	6.6b	7.1b	0.60	0.58	0.70	0.68	0.86	0.84
M4	3.4	5.6a*	4.6a	5.0a	0.59	0.61	0.71	0.72	0.83	0.85
SO4	4.2	5.3a	6.4b	5.4a*	0.61	0.61	0.70	0.70	0.87	0.86

Data are averages of 6 dates for each genotype and water regime combination. WW, Well-watered; DI, Deficit irrigation. Within each parameter and water regime, mean values followed by a different letter are significantly different at P<0.05; * means differences between water regimes, for that parameter and genotype.

2. End-of-season determinations

2.1. Oxidative stress tolerance

The oxidative parameters of leaf MDA and antioxidant activity was significantly affected by G and H_2O_2 by WR (ST2). Under WW conditions, significant differences among genotypes were observed for leaf MDA, H_2O_2 and antioxidant activity. Whereas, under DI, H_2O_2 showed no significant differences among genotypes. Overall, M4 and SO4 showed higher values for leaf concentration of MDA and H_2O_2, and higher antioxidant activity, compared to 110R and M1 (Table 4). Leaf MDA and H_2O_2 concentration were significantly decreased under DI compared to WW in SO4 genotypes, while in M4,

only the leaf H_2O_2 concentration was decreased. In 110R, the antioxidant activity was decreased from WW to DI conditions (Table 4).

2.2. Vine vegetative growth and water use efficiency

Overall, most biomass parameters were significantly affected by both G and WR, but also by GxWR interaction (ST2). Under WW regime, 110R had the longest main roots, followed by SO4, and plants from the M-rootstocks showed the shortest (Table 5). Nevertheless, under DI, there were no differences among genotypes. Only 110R significantly reduced main root length in response to water shortage (-33%).

Under WW irrigation, SO4 had the highest root mass, followed by 110R and M1, which had higher root mass than M4. No differences were found in this parameter under DI. Nevertheless, there was a significant reduction of 66% in root mass in plants of SO4 under DI compared to those from WW. Thus, under WW conditions, it seems that 110R was the genotype that developed a longer root system, while M-rootstocks were the ones that developed the least. Moreover, SO4 allocated more biomass in the roots under WW conditions, whereas under DI, it was the only genotype which significantly reduced this parameter (Table 5).

Differences in aerial biomass were found mainly under DI, whereas the opposite was observed underground (Table 5). Under DI, shoot mass and total biomass were the highest in 110R genotype, while SO4 had the lowest values, being intermediate from M-rootstocks. In addition, SO4 was the only genotype which significantly reduced these parameters between water regimes. Under WW conditions, only the total biomass of M4 was lower when compared to other genotypes. In the case of total leaf area, all genotypes reduced it in response to DI regime, without differences among them in this parameter (Table 5). Under DI conditions, SO4 developed less shoot mass per gram of developed root. This shoot-to-root ratio was only significantly lowered from WW to DI regime in M4 plants (Table 5).

Moreover, water content (WC) of 110R and M1 was higher when compared to M4 and SO4 under both WR (Table 5). Remarkably, in none of the genotypes WW plants showed differences in WC with their respective DI ones.

Regarding WUE_b, under WW conditions, 110R, M1 and SO4 genotypes had similar values (4.1 to 4.2 g dw L^{-1}), but were significantly higher compared to those from M4 plants (Ta-

Table 4. Malondialdehyde (MDA), Hydrogen peroxide (H_2O_2) and antioxidant activity in four rootstock genotypes (110R, M1, M4 and SO4) of well-watered (WW) and deficit irrigated (DI) plants.

Parameter	MDA (nanomol g^{-1} dw)		H_2O_2 (µmol g^{-1} dw)		Antiox. Act. (% inhibition)	
Factor	WW	DI	WW	DI	WW	DI
110R	43ab	39a	0.94a	0.77	32a	17a*
M1	40a	37a	1.10ab	1.07	49ab	55b
M4	54b	61b	1.34b	0.67*	60bc	68b
SO4	70c	60b*	1.04ab	0.58*	70c	64b

Data are averages of 6 plants for each genotype and water regime combination measured at the end of the experiment. WW, Well-watered; DI, Deficit irrigation. Within each parameter and water regime, mean values followed by a different letter are significantly different at P<0.05; * means differences between water regimes, for that parameter and genotype.

Table 5. Vegetative growth parameters (length of main roots, weights of roots and aerial tissues and total leaf area), water use efficiency in terms of total biomass (WUE_b) and water content of shoot tissues (WC) in four rootstock genotypes (110R, M1, M4 and SO4) of well-watered (WW) and deficit irrigated (DI) plants.

Parameter	Main root length (cm)		Root mass (g dw)		Shoots mass (g dw)		Total biomass (g dw)	
Factor	WW	DI	WW	DI	WW	DI	WW	DI
110R	99c	66*	67b	53	113	99c	180b	152c
M1	57a	60	63b	47	95	80b	158b	127b
M4	60a	59	41a	47	84	77b	125a	124b
SO4	77b	65	82c	49*	103	57a*	185b	105a*

Parameter	Total leaf area (m²)		Shoot-to-root mass		WUE_b (g dw L^{-1})		WC (%)	
Factor	WW	DI	WW	DI	WW	DI	WW	DI
110R	6.7	3.7*	1.7	1.9b	4.1b	8.5b*	28.1a	27.3a
M1	5.8	3.3*	1.6	1.7b	4.1b	7.3ab*	27.2a	27.2a
M4	6.9	3.6*	2.1	1.7b*	3.8a	6.1ab*	33.4b	33.2c
SO4	5.9	3.9*	1.4	1.2a	4.2b	4.4a	31.3b	30.4b

Data are averages of 6 plants for each genotype and water regime combination measured at the end of the experiment. WW, Well-watered; DI, Deficit irrigation. Within each parameter and water regime, mean values followed by a different letter are significantly different at P<0.05; * means differences between water regimes, for that parameter and genotype.

ble 5). Under DI conditions, WUE_b significantly increased in 110R, M1 and M4 compared to its respective WW treatment, while in SO4 plants it was not affected (see GxWR interaction in ST2). Thus, under water stress, SO4 showed significantly lower WUE_b than 110R, but these two genotypes did not show a significant difference with M1 and M4. Remarkably, a tendency to higher WUE_b in plants with higher WUE_i was found ($WUE_b = 1.5 + 0.07 * WUE_i$; $r^2 = 0.27$; *p* < 0.01).

3. Correlation matrix between variables

In Supplemental Fig. 1 (SF1) Pearson coefficient (%) of the linear regression between pairs of variables studied across the experiment is shown. Noteworthy are the significantly positive relationships between vegetative parameters (LA, shoot mass, root mass, total mass) and $Ψ_{stem}$ and gas exchange parameters at leaf level. Also, note the strong positive correlation between K_h (WB) and these vegetative parameters, and also with WU. Conversely, the relationship between K_h (WB) and WUE_i or WUE_b was significantly negative. Nonetheless, the relationship between K_h estimated by gas exchange and these variables were not significant, except for shoot mass and WUE_i.

Regarding chlorophyll content and oxidative damage, the positive relationship of Chl_{basal} with WUE_i and WUE_b is outstanding (SF1). Furthermore, Chl_{basal} was negatively related to K_h (WB) and WU, as well as to the shoot-to-root ratio. Antioxidant activity was also negatively related to both, Chl_{basal} and $Chl_{mid-apic}$. Lastly, H_2O_2 showed a negative relationship to root mass.

Discussion

1. Ecophysiological response of rootstocks to water stress

In this trial, the ecophysiological responses to soil water deficit of four *Vitis* sp. genotypes that can be used as grapevine rootstocks were studied. Two of these genotypes are the traditional rootstocks 110R and SO4, and two are new ones from the Italian breeding programs, M1 and M4 (Bianchi *et al.*, 2018). The results of this research indicate that the four genotypes were able to adapt to DI conditions imposed during three months. At the end of the experiment, all genotypes under DI conditions showed a good water status, despite of stress levels imposed by irrigation deficit, as shown by the fact that shoot water content was not reduced in the DI plants compared to WW plants (Table 5). In grapevines (*Vitis vinifera* L.), a water deficit of this level (35% ET_o) usually causes severe water stress, resulting in dehydration of the leaves, reduced growth, loss of yield, hydraulic failure, oxidative stresses, etc. as reported in previous experiment (Romero *et al.*, 2010; Dayer *et al.*, 2020; Gambetta *et al.*, 2020). In this experiment, plants did not experience shoot tissue dehydration (Table 5) despite reductions in vine water status ($Ψ_{stem}$) between WR (Table 1). This was likely due to different physiological mechanisms including K_h regulation, stomatal control, osmotic adjustment, and/or morphological changes in the different

plant tissues. Nevertheless, the strategies followed by each single genotype were different.

The decrease in water potential without changes in the water content of the tissues could indicate that these plants had an osmotic adjustment process, accumulating organic solutes to reduce the osmotic potential and so maintaining cell turgor and the potential gradient between the soil and the leaf (Rodríguez-Gamir *et al.*, 2010; Barrios-Masias *et al.* 2018). The genotype that least decreased the $Ψ_{stem}$ under DI was SO4, possibly indicating that this rootstock had a low capacity of hydraulic conductance regulation (Table 2) and/or high vegetative growth adjustment (Table 5) (Martínez-Vilalta and García-Forner, 2017). Moreover, when estimating hydraulic conductance by gas exchange, where substrate evaporation is not considered, SO4 was the only genotype that did not reduce K_h (GE) under DI compared to WW. This response is in agreement with that reported in 'Pinot gris' and 'Cabernet Sauvignon' vines grafted onto SO4 in comparison to other rootstocks (Koundouras *et al.*, 2008; Faralli *et al.*, 2020). It is also worth noting that M1, under water stress conditions, showed the lowest values of K_h calculated by both approaches. This suggests that this genotype may infer a greater regulation of water use under water limitation conditions (Table 2). In this sense, some authors have reported that different morphological-anatomical traits can also influence grapevine leaf water relations (Shtein *et al.*, 2017; Roig-Oliver *et al.*, 2020; Lupo *et al.*, 2021).

Another mechanism observed in this work is that the four studied genotypes were able to regulate g_s (Fig. 2), to decrease E and increase WUE_i (Table 1). In this regard, M1 showed lower g_s under DI than the other genotypes (Table 1), which suggests a greater capacity of stomatal regulation. The fact that g_s decreased without changes in shoot water content could suggest that stomatal closure was actively triggered by hormonal responses such as abscisic acid (ABA) rather than stomatal closure due to loss of turgor potential (Pou *et al.*, 2008; Gambetta *et al.*, 2020). Nonetheless, these authors have stated that stomatal regulation in grapevine is a complex and multilevel phenomenon, thus not correlated unequivocally with any single signaling factor (ABA, $Ψ_{stem}$ or hydraulic conductance) (Martínez-Vilalta and García-Forner, 2017). Since in our trial there were no differences in the g_s-to-$Ψ_{stem}$ relationships among genotypes (Fig. 3), it can be concluded that they had similar stomatal behavior in relation to the water status (Lovisolo *et al.*, 2016).

The decrease in g_s under DI obviously resulted in a decrease in A_N compared to WW conditions (Table 1), but the data reveal that this reduction was smaller than the reduction in g_s and therefore all genotypes increased WUE_i. This suggests that non-stomatal limitation did not occur in response to water DI in any of the genotypes, as evidenced by no differences found in the ratio c_i/c_a ratio between WRs (Chaves *et al.*, 2009; Shahid *et al.*, 2020). The absence of non-stomatal limitation in A_N reduction is also supported by chlorophyll fluorescence study as PSII parameter and its two components qP and Fv'/Fm' were not impaired by DI (Table 3). In this regard, Flexas *et al.* (2009) observed that leaf photochemistry of 110R genotypes was very resistant to water stress conditions.

The leaf MDA concentration and antioxidant capacity assessment, albeit only tested at the end of the experiment, supports that the four genotypes did not suffer damage under DI conditions (Table 4). Although the A_N-to-PSII ratio significantly decreased in DI plants compared to WW plants (data not shown), indicating that there was an excess of energy in the leaves that favors the formation of reactive oxygen species (ROS) (Zandalinas *et al.*, 2018). However, the leaf concentration of MDA was not increased, in none of the genotypes, suggesting that the plants were not damaged by oxidative stress, probably because their antioxidant activity was able to neutralize the ROS that would have been produced by the decrease in A_N (Fig. 2 and Table 4). In fact, regarding the oxidative metabolism products H_2O_2 and MDA, it was observed at the end of the trial that under WW conditions these values were increased in some genotypes. This was unexpected, because well-watered plants received more fertigation than DI ones, but appears to be inversely related to the chlorophyll content in the leaves (SF1). Hence leaves with lower CCI values showed higher H_2O_2 and MDA concentrations, and therefore higher, oxidative stress. The fact that, especially in basal leaves, the chlorophyll content was reduced under WW compared to DI suggests that some nutritional stress may have occurred. This was more clearly observed in 110R, M1 and M4 than in SO4 (Table 3). In general, a negative relationship was observed between Chl_{basal} content and LA (SF1), suggesting that excessive vigor under WW conditions promoted the translocation of nutrients from the basal leaves. However, SO4 appears to have less nutrient translocation capacity, as it did not reduce Chl_{basal} content in response to the water regime, but did reduce $Chl_{mid-apic}$ content in response to DI. Another variable that is inversely related to Chl_{basal} is the shoot-to-root ratio (SF1), which might be related to nutrient uptake capacity. These results suggest differences in nitrogen use efficiency among rootstock-genotypes under WW conditions. Based on chlorophyll content, 110R and M1 seem to be the most efficient under DI conditions (Table 3). This desirable feature of balanced leaf nutritional pattern was also reported for M1 when compare to M3, 1103P and 101-14 rootstocks (Zamboni *et al.*, 2016).

2. Vegetative development

The different adaptation mechanisms discussed above, made the total biomass of DI plants similar to that of WW in 110R, M1 and M4 genotypes (Table 5). Whereas in SO4, an overall decrease in total vegetative biomass was observed due to a significant reduction in both shoot and root mass. In fact, this genotype was one of the most vigorous genotypes under WW and however, the lowest under DI conditions. This decrease in growth could be due to the fact that this genotype reduced Ψ_{stem} the least under DI conditions, suggesting that SO4 reduces vegetative growth as a strategy to avoid water stress. Furthermore, the WC induced by SO4 was also higher than in 110R and M1 under DI (Table 5). This might be due to a lower osmotic adjustment or leaf morphology adaptation capacity compared to the other three genotypes to regulate vine water status. Thus, Iacono and Peterlunger (2000) suggested that the metabolic stability of plants growing under watering stress conditions depends on their ability to maintain high

photosynthetic activity and ABA flux under these conditions. Moreover, the accumulation in leaves of organic solutes such as proline or soluble sugars is fundamental in the tolerance to osmotic stresses (Toumi *et al.*, 2007; Degu *et al.*, 2019). Other trials study the ability of plants to adapt their morphological or anatomical characteristics under water stress conditions (Roig-Oliver *et al.*, 2020). For instance, Koundouras *et al.* (2008) reported that SO4, rootstock qualified as less adapted to limited water conditions than 1103P (qualified as drought tolerant), was able to maintain physiological mechanism and leaf water status at similar, or even higher rates with 1103P. This fact was possible due to the lower growth rate, lower leaf area and to possible adjustments of SO4 leaf structure. Other authors such as Gómez-del-Campo *et al.* (2003) observed that the stomatal density of each genotype could influence drought tolerance.

M4 tended to be less vigorous in both WRs, and stood out alongside M1 for its shorter root length. The 110R, however, showed the greatest root length under non-limiting water conditions, which might confer the greatest capacity to explore the soil in length (Table 5), although it significantly reduced it in response to DI. Despite this, 110R was the genotype with the higher vine performance under water stress conditions (Table 5). Thus, under DI, 110R tended to increase WUE_b compared to the other genotypes, although only differed significantly from SO4. In fact, the latter was the only genotype which surprisingly did not increase WUE_b in response to water deficit (Table 5).

In general, plants regulate their growth under water deficit conditions to reduce total transpiration and avoid tissue damage (Sadras *et al.*, 2017; Bonada *et al.*, 2018). Grapevines can also reduce the shoot-to-root ratio as an adaptation strategy (Alsina *et al.*, 2011; Zhang *et al.*, 2016; Chaves *et al.*, 2002). In our trial, this was the case of M4 under DI regime compared to WW conditions. Nevertheless, SO4 tended to show the lowest values of this ratio, and possibly for this reason it did not reduce its K_h (GE) under soil water deficit compared to WW (Table 2). Moreover, this genotype tended to have the highest root mass, but not the longest (Table 5), suggesting a dense root system (Fig. 4) that might favor the vine water uptake. This trait might further accentuate the differences under field conditions, where the explored soil volume is much higher than in pot conditions. In this regard, Alsina *et al.* (2011) found under field-grown Merlot grapevines, that differences in drought tolerance were due to the rootstock capacity to deep root proliferation during drought rather than to xylem anatomy or conductivity. For this reason, exploring rootstocks under pot conditions can provide for some insight into physiological responses to stress without the soil interaction. For instance, in 50 L containers, de Herralde *et al.* (2006) found that the hydraulic conductance in trunks of 'Tempranillo' was lower when grafted onto 110R than onto SO4. This is in agreement with our findings on hydraulic conductance between these two genotypes (Table 2). Nevertheless, the response of grapevine to water stress is very complex (Gambetta *et al.*, 2020), and there are other traits such as ABA that have been shown to play an important role in *Vitis* genotypes performance (Pou *et al.*, 2008). In view of our results, the strategies of the tested genotypes to cope with water stress conditions
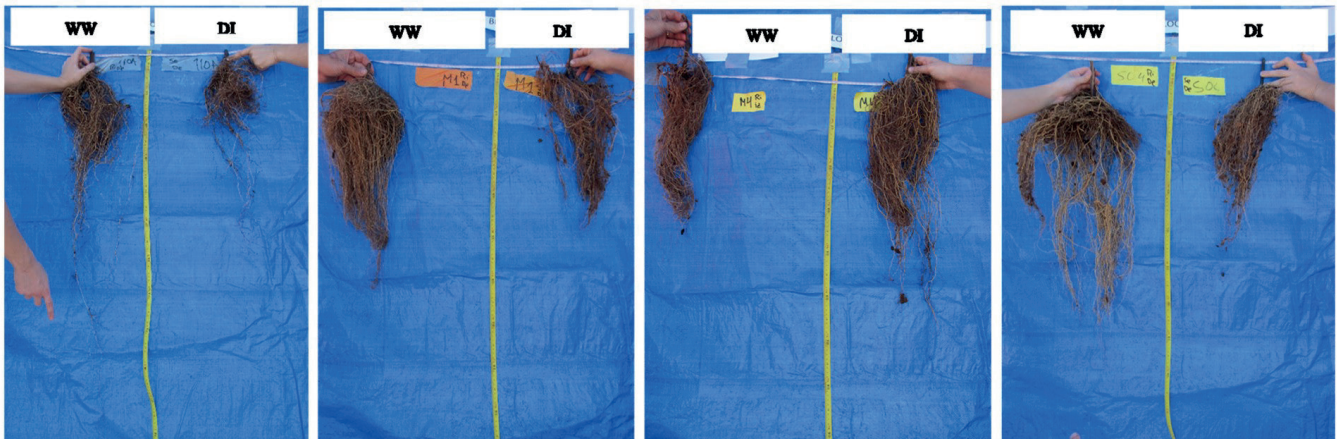
Fig. 4: Photographs of root morphology (main root length and root system structure) for 110R, M1, M4 and SO4 genotypes, from left to right, respectively, comparing water regimes (WW, well-watered and DI, deficit irrigated) for each genotype.

seem to be a trade-off between adaptation and avoidance (Chaves *et al.*, 2002). Thus, SO4 seems to be a water stress avoider by reducing vegetative growth in response to drought (Table 5), while the other three rootstocks would be adaptive. In fact, the physiological mechanisms against water stress displayed by 110R, M1 and M4 prevented reducing their total biomass in DI compared to WW conditions.

Among the four genotypes, 110R showed the most isohydric behavior in response to water deficit, based on WU results as shown by previous studies (Galmés *et al.*, 2007; Pou *et al.*, 2008). Contralily, SO4 behaved more anisohydrically (Faralli *et al.*, 2020), as this genotype reduces WU the least (Table 1) and also does not reduce K_h (GE) between WRs (Table 2). Therefore, SO4 strategy resulted in a lower WUE_b under water deficit conditions (Table 5), which does not seem an interesting way of conferring drought tolerance to the scion.

3. Implications on viticulture

Despite the experiment was carried out under potted and ungrafted conditions with the main aim of exploring different physiological traits in response to water restrictions by the evaluated genotypes, some viticulture implications from the obtained results could be discussed. This discussion can be made under the assumption that stomatal and thus photosynthesis regulation is mediated by hydraulic and hormonal signals from root-to-shoot (Franck *et al.*, 2020). However, this is known to be not so straightforward and that an interactive effect of the rootstock on the scion cannot be ruled out (Toumi *et al.* 2007; Marguerit *et al.*, 2012; Serra *et al.*, 2014; Bascuñán-Godoy *et al.*, 2017). Under no water limitations, there were clear differences in their performance, being M1 and particularly M4 the genotypes that reduced more biomass production. This suggests that the M-rootstocks might induce some vigor reductions to the scion and that their use could be suggested under certain terroirs where soil water holding capacity is very limited and/or vine growers might be willing to restrict vine growth searching for a more quality oriented production (Zhang *et al.*, 2016; van Leeuwen *et al.*,

2018; Faralli *et al.*, 2020). On the other hand, when the goal is to maximize production, rootstocks 110R and SO4 might be more suited given their superior performance in terms of biomass production in comparison with M1 and M4. This was found to be related to higher hydraulic conductance (Table 2). However, it is interesting to note that while the 110R genotype showed good capacity to withstand water restrictions in response to water stress with a reduction in total biomass of 16% compared with WW conditions, the SO4 genotype had a much larger reduction in growth. This suggests a lower tolerance of the latter to changing soil water availability, as it showed lower stomatal and water potential regulation capacity (Table 1). This is also supported by WUE_b values reported which were the lowest in SO4 rootstock under DI conditions (Table 5). Therefore, SO4 is confirmed as an uninteresting rootstock under soil water deficit conditions (Koundouras *et al.*, 2008; Meggio *et al.*, 2014; Merli *et al.*, 2016), because it could reduce A_N and thus impair yield or stop berry ripening (Medrano *et al.*, 2003). The reductions in root biomass under water deficit conditions (Table 5), together with its inability to regulate hydraulics (Table 1 and 2), suggest that in the face of prolonged drought it could reach thresholds of water stress in which photosynthesis does not allow the accumulation of photo-assimilates to the grape (Romero *et al.*, 2010). This is consistent with SO4 being a rootstock in viticultural areas where available water is not a very limiting factor (Ollat *et al.*, 2016; Marín *et al.*, 2021). On the other hand, the variations in growth due to water stress from both M1 and M4 genotypes was minimal and they were able to maintain WUE_b values similar to 110R. This is a feature of importance from a vineyard sustainability point of view where it is also interesting to compare the rootstock performance in terms of whole vine WUE. In this sense, Merli *et al.* (2016) found that under water stress, the M4 rootstock improved whole-canopy water use efficiency and berry composition of 'Sangiovese' grapevines compared to grafted onto SO4. However, in our trial, under WW conditions, M4 showed the lowest WUE_b (Table 5). This would suggest against its use to maximize yields, but it might be interesting to improve grape ripening as other authors observed (Merli *et al.* 2016).

Conclusions

This study shows that vine growth is dependent on the substrate water availability and on *Vitis* genotypes. Overall, SO4 and 110R were the most vigorous genotypes, while M1 and M4 might be considered less vigorous. Therefore, under conditions of high potential vine growth, the latter genotypes might be employed as a tool to limit vine vigor when the target is to improve grape and wine composition. Under conditions of limited soil water availably the four genotypes adapted well, but following different strategies. Under water deficit, 110R was the one that best maintained vine performance while reducing water use, thus improving WUE_b, whereas SO4 clearly reduced both shoot and root growth and WUE_b. SO4, in fact, seems to be a water stress avoider by reducing vegetative growth in response to drought, likely due to a low capacity of hydraulic and stomatal regulation. Conversely, the M-rootstocks here explored, particularly M1, showed an overall lower hydraulic conductance, which might confer some advantages under deficit irrigation conditions by inducing a more conservative water use. In any case, the differential vine growth and physiological responses observed could not be fully explained just by changes in water use and leaf gas exchange patterns, suggesting that water stress responses are more complex, involving differences in oxidative stress tolerance, nutrient translocation capacity, shoot-root balance, etc. The results obtained in this work with ungrafted plants point to the main mechanisms driving stress-tolerance, which should be now corroborated in field studies by grafting the explored genotypes with different grapevine varieties.

Acknowledgements

Conflicts of interest

The authors declare that they do not have any conflicts of interest.

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