

Ecophysiological performance of *Vitis* rootstocks under water stress

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Abstract

An ecophysiological assessment of the conventional 110-Richter (110R) and SO4, and the new M1 and M4 rootstocks under water deficit was carried out in ungrafted potted plants. Water potentials and gas exchange rates of these *Vitis* genotypes under deficit irrigation reached severe water stress levels causing only slight effects on leaf chlorophyll content. Gravimetric differences in daily water use were observed among genotypes, leading to differences in the biomass of root, shoot and leaf. The sensitivity of the genotypes to water deficit was different, with SO4 exhibiting the greatest reduction in biomass while 110R showing the lowest. Remarkably, under water deficit, SO4 reached the least negative stem water potential, while M1 reduced gas exchange the most. In addition, SO4 also showed the highest and lowest hydraulic conductance values. Our results suggest that the evaluated genotypes follow different adaptation strategies to water deficit. Differences in water use among genotypes were not only attributable to differences in stomatal regulation at the leaf level, but also to hydraulic regulation and nutrient translocation capacity. These differences in ecophysiological performance among potted *Vitis* rootstocks encourage to confirm this under field conditions and how they translate to the scion.

Introduction

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 while respecting local traditions of the wine sector (Ollat et al. 2016; van Leeuwen and Destrac-Irvine 2017; Romero et al. 2018; Marín et al. 2021). The role of genetic material, and in particular of the rootstock, in improving vine performance under water stress conditions is poorly understood (Medrano et al. 2015; Zhang et al. 2016).

Rootstocks can induce tolerance to biotic and abiotic limiting factors such as drought, salinity, or nutritional deficiencies (Serra et al., 2014; Bianchi et al., 2018). Rootstocks influence stomatal conductance, transpiration and leaf photosynthesis and thus vine vigor (Gambetta et al., 2012), leaf and bunch mineral composition (Zambioni et al., 2016), and yield and grape composition (Romero et al., 2018). However, currently, and according to Serra et al. (2014), only 10 rootstocks are used for about 90% of the grapevine genotypes established around the world. This limited genetic background is a great problem to select the optimum rootstock for each variety and climatic scenarios (Riaz et al., 2019). Therefore, breeding programs of new rootstock genotypes, with more diverse and better performances, are needed for improving the sustainability of the grape industry (Serra et al., 2014).

In the present experiment, the morphological and physiological responses to water stress of two novel genotypes, M1 and M4 (Bianchi et al., 2018), were compared to two traditional rootstocks widely used in

viticulture, 110R and SO4 (Ollat et al., 2016; Galmés et al. 2007; Pou et al., 2008). The objective was to know the relative tolerance to the drought condition among them and, identifying 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) (35% ET_0) and well-watered (WW). Vine performance and physiological responses of the plants, including biomass allocation, stem water potential, leaf gas exchange parameters, hydraulic conductance and leaf chlorophyll content were measured.

Materials and methods

The trial was conducted with ungrafted grapevine plants (*Vitis* species) in 25 L pots located in a greenhouse at the CEBAS experimental field (Santomera, Murcia, Spain). The experimental design consisted in four different rootstocks genotypes (G) subjected to two different water regimes (WR): well-watered (WW) and deficit irrigation conditions (DI). Each combination rootstock x water regime had a total of 12 plants, for a total of 96 experimental plants. The rootstocks used were: Richter-110 (*V. berlandieri* x *V. rupestris*), and SO4 (*V. berlandieri* x *V. riparia*), widely used in world viticulture and, two recently bred ones: M1 (106-8 (*V. riparia* x *V. cordifolia* x *V. rupestris*)) x (*V. berlandieri*)), and M4 [(*V. vinifera* x *V. berlandieri*) x *V. berlandieri*].

Well-watered plants were irrigated to field capacity throughout the experiment. Deficit irrigation plants were watered daily at 35% of the reference evapotranspiration (ET_0). All plants received Hoagland nutrient solution at 50%, applied during irrigation in the whole experiment. Two shoots per vine were directed upward along the catch wires. Shoots were trimmed when it reached 2 m length. 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.

Vine water use was estimated by daily water balance (6 times/season). Water use over the experiment was calculated as the average daily use times the number of days from the 5th September to 19th October (WU, L/plant). Grapevine water status was determined with a Scholander pressure chamber as midday stem water potential (Ψ_{stem}). Ψ_{stem} was measured weekly from September to October on one leaf per plant in two plants for combination G x WR per block (n = 6).

Leaf net photosynthesis (A_N ; $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance (g_s ; $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) and transpiration rate (E; $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) were measured with a portable infrared gas analyzer LCPro+ Portable (ADC, Bioscientific, Ltd, UK). Gas exchange determinations were measured between 10.00 and 12.00 h solar time in the same days and the same plants as the Ψ_{stem} . 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 $K_h = E/(\Psi_{soil} - \Psi_{stem})$ (Tsuda and Tyree, 2000).

Leaf chlorophyll content was measured in fully developed basals and in medium and apical leaves in all orientations by a portable CI-01 chlorophyll content meter (Hansatech Instruments Ltd., Norfolk, UK) in 6 plants of G x WR combinations.

At the end of the trial (19th October), 48 plants (2 plants of each G x WR combination; n = 6) were used for weighing the two main shoots of each plant and its secondary shoots. Also, the whole root systems of these plants were taken out of the pots, cleaned with tap water, weighed and the length of the main root was measured. Both, the aerial part and the roots per plant were placed in an oven until constant weight (70 °C) to obtain dry weight. The 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 regime. 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).

The statistical analysis was carried out by means of a three-way analysis of variance (ANOVA) using the "Statgraphics Centurion XVI" package version 16.0.07 (StatPoint Technologies, Inc., Warrenton, VA, USA). 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 x WR were detected for many of the variables considered, data are shown in average of G x WR combination. Mean separation was carried out via Duncan's test (significant at $p < 0.05$).

Results and discussion

The ecophysiological responses to water deficit of four grapevine rootstocks were studied. Overall, the Ψ_{stem} showed a significant effect of WR and D, but not of G. Nevertheless, there was a significant GxWR interaction.

In fact, pooling data across the experimental period there were not statistically significant differences among genotypes in vine water status under WW, but it did 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. This vine water status corresponded to leaf net assimilation rates (A_N) ranging from 10-12 $\mu\text{mol}/\text{m}^2\text{s}^{-1}$ and stomatal conductance (g_s) around 0.20 to 0.25 $\text{mmol}/\text{m}^2\text{s}^{-1}$, without showing significant differences among genotypes (Table 1).

Table 1. Water relations, hydraulic conductance and gas exchange parameters across the experiment.

Parameter	Ψ_{stem} (MPa)		A_N ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)		g_s ($\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)		E ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)		K_h ($\text{mmol H}_2\text{O MPa}^{-1} \text{ m}^{-2} \text{ s}^{-1}$)		WU (L plant^{-1})	
	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*	7.4	4.5b*	44.4b	18.2a*
M1	-0.64	-1.26a*	8.7	4.7a*	0.210	0.078a*	2.8	1.5a*	5.7	2.6a*	38.8ab	18.6a*
M4	-0.65	-1.19ab*	8.9	7.3b*	0.201	0.120b*	2.8	2.0b*	7.1	4.4b*	33.1a	20.5b*
SO4	-0.69	-1.06b*	9.1	6.8b*	0.226	0.152b*	3.2	2.3b*	7.3	5.9b	44.6b	26.1b*

WW, Well-watered; DI, Deficit irrigation; Ψ_{stem} , stem water potential; A_N , leaf photosynthesis rate; g_s , stomatal conductance; E, leaf transpiration; K_h WB, hydraulic conductance and WU, water use. 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.

In general, the SO4 vines showed significantly less negative Ψ_{stem} values than the 110R and M1 genotypes (Table 1), differing from M4. This might be indicating that SO4 has a low capacity of osmotic adjustment compared to the other genotypes (Barrios-Masias et al. 2018). In relation to gas exchange parameters under DI, the M1 vines stand out by lower g_s , A_N and E values than the other genotypes (Table 1), which suggests a greater capacity of stomatal regulation. Root to stem hydraulic conductance was significantly affected by G, WR, D and WRxD. The K_h showed no significant differences among genotypes under WW conditions, under DI however, the SO4 did not reduce its values between WR in contrast to that observed in 110R, M1 and M4 (Table 1). Differences between 110R and SO4 are in agreement with previous findings (de Herralde et al., 2006).

Differences in WU among rootstock genotypes were consistently found under both water regimes. 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. These results are in agreement with previous studies carried out in 110R which showed that this genotype to behaved isohydrically in response to water deficit (Galmés et al., 2007; Pou et al., 2008).

Chlorophyll content was significantly affected by G and D in both basal and medium-apical leaves. The WR had a significant effect on basal chlorophyll content and the GxWR on medium-apical leaves. This implies that water stress affected $\text{Chl}_{\text{mid-apic}}$ in a genotype-dependent manner (Table 2). Under DI conditions, the leaf $\text{Chl}_{\text{mid-apic}}$ concentration was not affected in 110R, M1 and M4, but it was significantly reduced by 15.62% in SO4. Overall, the 110R and M1 genotypes showed the highest values of this parameter. The leaf $\text{Chl}_{\text{basal}}$ was higher under DI than WW in all genotypes, being this increase higher for 110R, M1 and M4 (61%, 54%, 65%, respectively, compared to its respective WW ones) than for SO4 genotype (26%). Thus, under DI conditions, the highest leaf $\text{Chl}_{\text{basal}}$ was found again in 110R and M1 genotypes, whereas, under WW, $\text{Chl}_{\text{basal}}$ did not differ among genotypes (Table 2).

Overall, biomass parameters were significantly affected by both G and WR, but also by GxWR interaction. Under the WW irrigation, SO4 had the highest root mass, followed by 110R and M1, which had higher root mass than M4 (Table 2). Under DI regime, no differences were found in this parameter. Nevertheless, SO4

rootstock allocated more biomass in the roots under WW conditions. However, under DI, it was the only genotype which significantly reduced this parameter (Table 2). Interestingly, differences in shoot mass were found mainly under DI, whereas the opposite was observed underground (Table 2). Under DI, both shoot and total mass were the highest in 110R genotype, while SO4 showed the lowest values, being intermediate those from M-rootstocks. In addition, the SO4 was the only genotype which significantly reduced biomass between WW and DI treatments (Table 2).

Regarding WUE_b, the 110R, M1 and SO4 genotypes had similar values under WW conditions, but significantly higher compared to that from M4 plants. Under DI conditions, WUE_b significantly increased in 110R, M1 and M4 compared to its respective WW treatment, while in SO4 plants was not affected. Thus, under DI, the SO4 showed significantly lower WUE_b than 110R, but these two genotypes did not show significant difference with M1 and M4. Therefore, SO4 presents bad behavior under soil water deficit conditions, because it might reduce yield and also impair or stop berry ripening

Table 2. Chlorophyll content in apical shoots (apical) and fully expanded leaves (medium) in SPAD units in four *Vitis* cultivars.

Parameter	Chl _{basal}		Chl _{mid-apic}		Root mass (g)		Shoots mass (g)		Total biomass (g)		WUE _b (g dw L ⁻¹)	
	WW	DI	WW	DI	WW	DI	WW	DI	WW	DI	WW	DI
110R	4.6	7.4b*	7.9c	7.5b	67b	53	113	99c	180b	152c	4.1b	8.5b*
M1	4.6	7.1b*	6.6b	7.1b	63b	47	95	80b	158b	127b	4.1b	7.3ab*
M4	3.4	5.6a*	4.6a	5.0a	41a	47	84	77b	125a	124b	3.8a	6.1ab*
SO4	4.2	5.3a	6.4b	5.4a*	82c	49*	103	57a*	185b	105a*	4.2b	4.4a

WW, Well-watered; DI, Deficit irrigation; Chl_{basal}, chlorophyll content in basal leaves; Chl_{mid-apic}, chlorophyll content in medium and apical leaves; WUE_b, water use efficiency in terms of total biomass. 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.

Conclusion

This study shows that The *Vitis* genotypes employed differed in vine growth in response to water availability. Overall, SO4 and 110R were more vigorous genotypes than M1 and M4. Therefore, under conditions of high potential vine growth, the latter genotypes could be employed as a tool to limit vine vigor when the target is to improve grape and wine composition. Under water deficit, 110R rootstock-maintained vine performance while reducing water use, thus improving WUE_b, whereas the SO4 clearly reduced both shoot and root growth and WUE_b. The SO4, in fact, showed a lower capacity of hydraulic and stomatal regulation. Conversely, the M-rootstocks here explored, particularly M1, showed an overall lower hydraulic conductance. The different strategies displayed by the ungrafted rootstocks should be consider for possible genetic choices to cope with water deficit according to the winegrower's production objectives. The results obtained here should be now corroborated in field studies.

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