Local ancient grapevine cultivars to face future viticulture

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Abstract

Among the different strategies to cope with the negative impacts of climate change (CC) on viticulture, the exploitation of genetic diversity is one of the most promising to adapt to new conditions and maintain wine production and quality. One of the biggest concerns in the context of CC is to improve water use efficiency (WUE). In this way, the use of genotypes that present a better response to drought and high WUE is a key issue. In this work, physiological performance analysis was conducted to compare the water deficit stress (WDS) responses of local and widespread grapevines cultivars. Leaf gas exchange, WUE at different levels (leaf (WUEi) and long-term WUE ($\delta^{13}C$)) and water relations parameters such as plant hydraulic conductivity (Kh) were determined in plants under well-watered and WDS conditions alongside assessment of the levels of foliar hormones concentrations. Results denote that local cultivars displayed better physiological performance under WDS as compared to the widely-distributed ones. The results confirmed that hormone signals are produced before hydraulic signals and corroborate the hypothesis that better stomatal control mediated by abscisic acid (ABA) signalling, allows increasing WUE under drought as occurred in the local Callet cv.

Introduction

Climatic change (CC) is affecting grapevine growth and development, with clear effects on plant production and grape quality. The exploitation of genetic diversity is one of the options for adapting to new conditions and maintaining wine production and quality (Fraga, 2019; van Leeuwen et al., 2019). The Vitis International Variety Catalogue (VIVC, http://www.vivc.de/) lists around 24,500 accessions which include cultivars, breeding lines, and different *Vitis* spp. Nevertheless, few varieties such as Cabernet Sauvignon, Merlot, Chardonnay, Sauvignon Blanc and Syrah dominate vineyards worldwide while many cultivars, largely cultivated in the past, are now minority cultivars of each winegrowing region. Physiological and agronomic evaluation is the first step to recovering these minority cultivars from extinction and testing their capacity to CC adaptation. The evaluation of local cultivars from the Balearic Islands started in 2001. Since then, different studies evidenced better performance under water deficit stress of some local cultivars in terms of their photosynthetic characteristics, water relations, WUE and metabolic profile (Bota et al., 2001; 2016; Florez-Sarasa et al., 2020). Two of the local cultivars evaluated, Callet and Escursac, showed the most differentiated behaviour in the different studies (higher net photosynthesis, higher WUE, no reduction in Kh, among other responses to drought). From these previous results, we hypothesised that high Kh and better stomatal control could be responsible for better WUE under water deficit and that hormone levels and their variations could explain this cultivar-specific drought response. For this reason, in this work, we have studied the water deficit stress (WDS) responses in the two local cultivars (Callet and Escursac), a reference Spanish cultivar (Garnacha) and two widely-distributed ones (Merlot and Syrah) by measuring several ecophysiological parameters in combination with hormone profiling analysis.

Materials and methods

Five grapevine cultivars were chosen to perform the present study; two red local ancients (pre-*Phylloxera*) cultivars: Escursac and Callet, the Spanish cultivar, highly reputed as drought-tolerant, Garnacha and two widespread French cultivars: Merlot and Syrah. The experiment was performed in the summer of 2016 at the experimental field site of *Universitat de les Illes Balears* (Spain). Plants were grown outdoors in 15 L pots filled with organic substrate and perlite mixture (3:1). Four plants per cultivar were selected for each irrigation treatment, control (WW) and water deficit stress (WDS). Control plants were irrigated at field capacity during the experiment, the WDS treatment was established by allowing pots to dry until the water content in the pot rounded 50% of field capacity. The plant water status was estimated by pre-dawn water potential (Ψ_{PD}).
Gas Exchange measurements: Leaf net photosynthesis (A_N), stomatal conductance (g_s) and transpiration rate (E) were measured in four leaves per cultivar. Measurements were done between 9:00 and 12:00 h using an infrared open gas exchange system (Li-6400, Li-cor Inc., Lincoln, Nebraska, USA). Intrinsic water use efficiency (WUEi) was calculated as the ratio between A_N and g_s.
Leaf carbon isotope composition $\delta^{13}C$: Carbon isotope composition in leaf dry matter was measured as in Tomás et al., 2012.
Hormone analysis: Phytohormones were extracted and analyzed according to Großkinsky et al. (2014).
Statistical analysis: Effects of the factors' water deficit stress and cultivar on the response variables were tested by a two-way analysis of variance (ANOVA). The distribution of the residual was graphically revised and a logarithmic transformation of the response variable was used when the residual distribution was not homogenous or normal. Means ± standard errors (SE) were calculated, and when the F ratio was significant ($p < 0.05$), a posteriori T-test was applied. Statistical analyses were carried out using statistical software JMP 10 (SAS).

Results and discussion

Under WW conditions, little variation in net photosynthesis (A_N) and stomatal conductance (g_s) was observed among genotypes. The water deficit imposition resulted in a significant decrease in A_N and g_s in all cultivars (Table 1). In a recent study, the local cultivars Callet and Escursac showed higher A_N under WDS than the widespread Merlot and Syrah (Florez-Sarasa et al., 2020). However, we did not observe this effect with similar A_N reduction among cultivars. Callet was the cultivar with higher g_s reduction (72%), but only Syrah reduced significantly the Ψ_{PD} (Tables 1, 2). Despite the expected reductions in photosynthesis in all cultivars, only the reputed drought-tolerant Garnacha and the local cultivar Callet increased the WUEi under WDS (Table 2). Moreover, only Callet showed higher long-term WUE ($\delta^{13}C$) under WDS than under WW conditions.
Merlot and Syrah significantly reduced their plant hydraulic conductance (Kh) under WDS, while local ones and Garnacha did not (Table 2). Moreover, a significant correlation was observed between WUEi and Kh (data not shown) being Garnacha and Callet the cultivars with higher WUEi under WDS with no reductions in Kh. The hydraulic conductance is one of the key physiological factors in stomatal regulation in grapevine and a cultivar dependent relationship between WUEi and Kh has been demonstrated before (Martorell et al., 2015). Florez-Sarasa et al. (2020), also reported non-reductions in plant Kh in Callet and Escursac under WDS.
Between the two local cultivars, Callet, with similar or even higher reductions in A_N, g_s and Kh than Escursac, increased both WUEi and $\delta^{13}C$ under WDS (Table 2). In a previous study (Bota et al., 2001), Escursac was found to be an interesting cultivar to maintain reasonably high carbon assimilation and low water consumption, resulting in high WUE both under drought and irrigation. However, in our experimental results, in concordance with those presented by Florez-Sarasa et al. (2020), Escursac did not show a large WUE increase in WDS conditions (Table 2).
Concerning phytohormonal signalling, WDS induced changes in the most important endogenous hormone levels (Figure 1). A strong decrease of *trans*-Zeatin (tZ) content in all the cultivars was observed. This result corroborates the previously described important role of Cks in regulating the early response to drought (Granda et al., 2011). On the other hand, the role of ABA as a stress signal was widely investigated in grapevine (Marusig and Tombesi., 2020). Under WDS all cultivars presented leaf ABA accumulation (Figure 1). The tZ and ABA results confirmed that hormonal signals occur earlier than hydraulic signals, as only Syrah showed a significant reduction in Ψ_{PD} under WDS. In Callet, minimal Ψ_{PD} variations produced similar changes in tZ and ABA

concentrations than in Syrah (table 2). But, only the local cultivar increased both WUEi and $\delta^{13}C$, and do not reduce Kh under WDS.

Positive significant correlations were found between WUE parameters (WUEi; $\delta^{13}C$) and ABA concentration (data not shown). These results corroborate the hypothesis that better stomatal control in Callet mediated by ABA and maintained Kh allows increasing WUE under drought in this cultivar.

While all cultivars followed the same response pattern for tZ and ABA, the response for jasmonic acid (JA) and salicylic acid (SA) was different among cultivars (Figure 1; Table 2). Only the two local cultivars increased the JA concentration being significant in Escursac. An antagonist pattern was observed for SA, with significant reductions for Escursac and increases in Merlot cultivar under WDS (Figure1; Table 2). It has been reported in several studies that JA signalling pathways are associated with the alleviation of drought stress (revised by Ali and Baek, 2020). On the other hand, the role of SA as a defence tool has been extensively evaluated, however its effect on drought tolerance still needs more investigation. In any case, it seems that the hormone cross talk is a mechanism to increase the survival of plants under drought (Ullah et al., 2018).

Conclusion

The results confirmed the better water use efficiency under drought of the local cultivar Callet as compared to widespread cultivars. Plant hydraulic conductance (Kh) was not significantly reduced in local cultivars and Garnacha but decreased in the wide-distributed cultivars (Merlot and Syrah) under WDS. The role of major phytohormones (tZ, ABA, JA and SA) could explain the cultivar differences observed between Callet and Escursac and widespread cultivars. Tighter ABA-mediated control of stomatal aperture under drought is probably responsible for the increased WUE in the local cultivar Callet. This kind of behaviour under drought may confer better adaptation to future expected climatic conditions in the Mediterranean area.

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Table 1. Pre-dawn water potential (□$_{PD.}$ MPa) in control (WW) and water stressed plants (WDS). Data are means ± S.E. of n= 4 plants per cultivar and treatment. Different letters indicate significant differences between groups ($p < 0.05$ a posteriori t-student).

Cultivar	A_N (µmol$CO_2 \cdot m^{-2} \cdot s^{-1}$)		g_s (mol $H_2O \cdot m^{-2} \cdot s^{-1}$)		$\Psi_{PD.}$ (MPa)	
	WW	**WDS**	**WW**	**WDS**	**WW**	**WDS**
Callet	18.28 ± 1.04c	8.06 ± 1.76a	0.34 ± 0.05e	0.09 ± 0.02a	-0.25 ± 0.03bc	-0.39 ± 0.03ab
Escursac	18.05 ± 0.94c	7.85 ± 0.46a	0.31 ± 0.02de	0.12 ± 0.02ab	-0.29 ± 0.04bc	-0.38 ± 0.04abc
Garnacha	15.71 ± 1.52bc	7.01 ± 0.88a	0.25 ± 0.03cd	0.08 ± 0.02a	-0.37 ± 0.03abc	-0.45 ± 0.01a
Merlot	15.73 ± 1.26bc	5.14 ± 0.36a	0.23± 0.03bc	0.06 ± 0.00a	-0.36 ± 0.04abc	-0.45 ± 0.05a
Syrah	17.38 ± 2.21c	6.63 ± 0.68a	0.29 ± 0.024de	0.09 ± 0.02a	-0.28 ± 0.02bc	-0.48 ± 0.05a

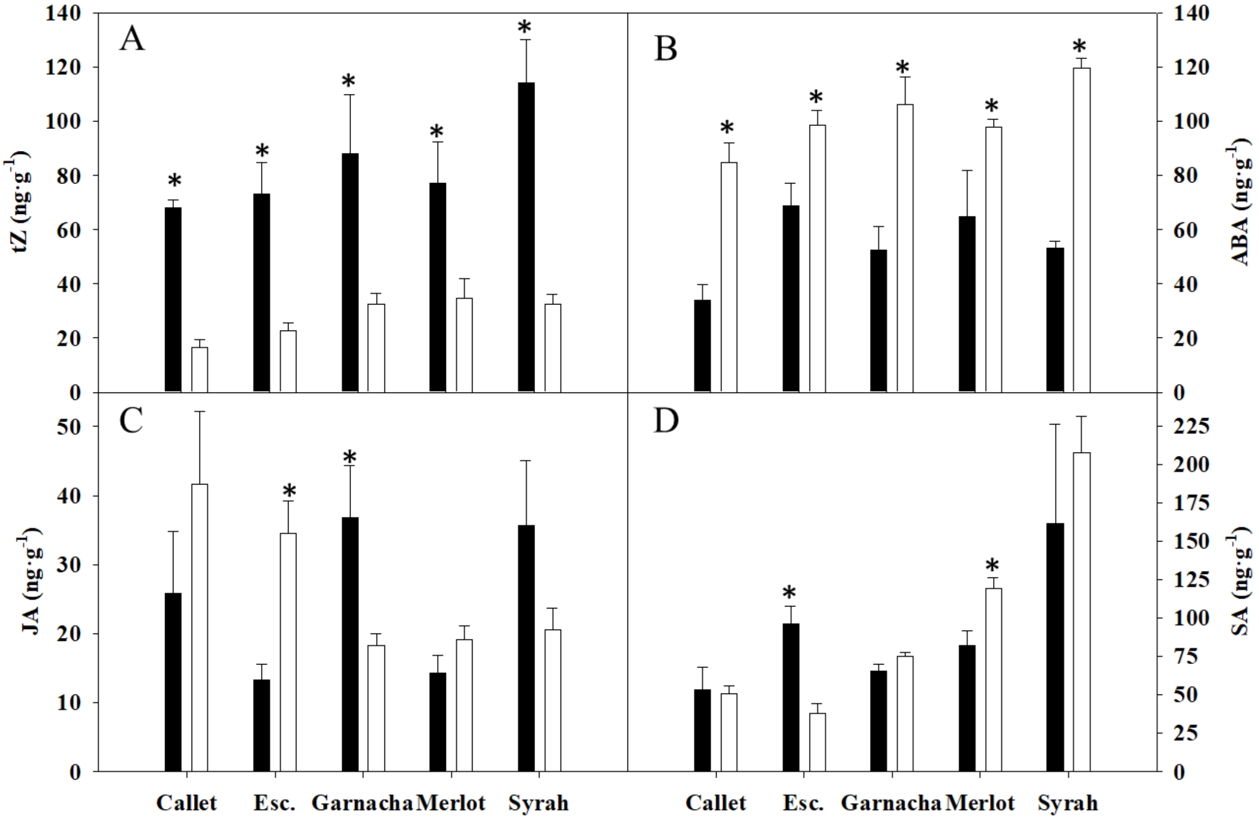

Figure 1. The *trans*-Zeatin (tZ) (A), Abscisic acid (ABA) (B), Jasmonic acid (JA) (C) and Salicylic acid (SA) (D) content in five grapevine cultivars under well waters conditions (black bars) and water deficit stress (white bars). M±SE of four plants per treatment. Significant differences between treatments are represented by asterisks (p < 0.05 a posteriori t-student).

Table 2. Fold-changes heatmap between water deficit stress (WDS) and well-watered (WW) data of the physiological traits and hormone concentrations in all grapevine cultivars. Asterisks denote significant changes (p< 0.05 a posteriori t-student) between WDS and WW conditions.

Cultivar	Physiological traits							Hormones			
	A_N	g_s	E	Ψ_{PD}	Kh	$\delta^{13}C$	WUEi	tZ	ABA	JA	SA
Callet	-0.60*	-0.72*	-0.68*	0.57	-0.58	-0.05*	0.56*	-0.76*	1.48*	0.62	-0.04
Escursac	-0.58*	-0.57*	-0.68*	0.33	-0.47	-0.03	0.19	-0.69*	0.43*	1.61*	-0.60*
Garnacha	-0.54*	-0.60*	-0.69*	0.23	-0.52	-0.05	0.49*	-0.63*	1.02*	-0.50*	0.15
Merlot	-0.64*	-0.67*	-0.77*	0.24	-0.74*	-0.01	0.18	-0.55*	0.51*	0.34	0.45*
Syrah	-0.49*	-0.57*	-0.70*	0.71*	-0.64*	-0.04	0.24	-0.71*	1.24*	-0.43	0.29

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