CONSISTENCY OF THE HYDRAULIC TRAITS AND STOMATAL RESPONSES IN GRAPEVINES WITH CONTRASTING HYDRAULIC VULNERABILITY

Authors:Luis VILLALOBOS-GONZÁLEZ[1], Constanza QUINTANA[1], Dayna DONAIRE[1], Mariana MUÑOZ-ARAYA[1], Nicolás FRANCK, Claudio PASTENES[1*]

[1]*Universidad de Chile, Facultad de Ciencias Agronómicas.*

*Corresponding author:cpastene@uchile.cl

Abstract:

Context and purpose of the study - Different from wild species in arid and semiarid conditions, cultivated species are very sensitive to drought and, beyond some stress thresholds, food production is not possible. It is therefore important to gain further knowledge on the responses of plant species of agricultural importance to realistic water shortage extents, and their consistencies. A valuable model for water stress studies has been the grapevine (*Vitis vinifera* L.), a species with a high variability in their stomatal sensitivity to water stress. In contrast to usual agricultural practices, grapevines for oenological purposes are grown under controlled water stress conditions.

Material and methods - In the abovementioned context, we aimed to study the physiological responses to a progressive, not severe (Ψ_{pd} > -1MPa), water deficit in the grapevine varieties Syrah and Carménère, in two consecutive season using different sites each year, and with different row orientation. We assessed a) the relationship between the water status of plants and the stomatal responses to water availability; b) the hydraulic traits derived from Ψ isotherms (pressure vs volume curves); c) the impact of the water stress on the hydraulic traits derived from the pressure vs volume curves and on photosynthetic responses; d) the stomatal sensitivity to ABA (only on one of the study sites) and e) their stem hydraulic vulnerability in relation to xylem characteristics.

Results – Acording to the Ψ_{leaf}/Ψ_{pd} relationship (σ), and contrary to various previous reports, we found Syrah to be an isohydric grapevine variety, while Carménère, an emblematic variety cultivated in Chile, behaved as anisohydric. Syrah resulted to be more variable in terms of σ, g_{s50} and g_{s12} (the ψ_{pd} upon which stomata is reduced down to a 50% and 12%), π_0 and ψ_{tlp} (the ψ at full turgor and the ψ at turgor loss point), likely associated to the higher genetic variability of Syrah compared to that of Carménère. Also, Carménère, the anisohydric variety was capable of osmotic adjustment and was more sensitive to ABA at the stomatal level, two traits typical of anisohydric species, contrary to that observed in Syrah. Even though the capacity to maintain stomata open, theoretically, would imply a lower energy load into the photosynthetic systems, both varieties reduced their photosynthetic capacity up to a similar extent upon drought. Finally, Syrah, despite having wider xylem vessels than Carménère, is less vulnerable to cavitation, and had a lower hydraulic safety margin, typical of isohydric species.

We will discuss our results in terms of the genetic variability of the varieties regarding the consistency of their hydraulic responses, the importance of the environment, the degree of isohydry in relation to stomatal responses to critical thresholds as well as drought resistance, and the implications for photosynthesis in the long term.

Keywords: ABA sensitivity, anisohydry, drought resistance, grapevine, stomatal conductance.

1. Introduction

A divergent behaviour on the regulation of the midday leaf water potential (Ψ_{md}) has been described, termed as isohydric and anisohydric (Tardieu et al. 2015), and interpreted as the result of a distinct stomatal control. While isohydric plants are conceived as conservative in their water balance by reducing their stomatal conductance (g_s) and therefore maintaining the leaf water potential at midday (Ψ_{md}), regardless of the soil water content (Steudle 2000, Bucci et al. 2004), anisohydric plants have been defined as less responsive, progressively reducing their Ψ_{md} upon drought (Tardieu and Simonneau 1998, Sade et al. 2012). From discrepancies found in the stomatal responses to water availability and the Ψ_{md}, the degree of isohydry has been proposed to be assessed by σ, the slope of the variations between Ψ_{md} and the pre-dawn water potential (Ψ_{pd}) (Martínez-Vilalta et al. 2014).

Grapevine varieties have already been classified along the iso/anisohydric continuum, but with inconsistencies thought to result from an eventual plant and environment interaction rather than an intrinsic plant property (Lovisolo et al. 2010; Schultz 2003, Hochberg et al. 2013, Charrier et al. 2018). Also, inferences on the water stress resistance and physiological responses regarding the Ψ_{md} regulation have been made assuming the degree of isohydry from literature. Therefore, in order to prove an eventual intrinsic response of Carménère (an emblematic variety in Chile) and Syrah to drought, we have assessed the effect of irrigation withholding in two consecutive seasons using different sites each year, i.e. under different environmental conditions, row orientations, clones and rootstocks, assessing physiological responses related to stress resistance.

2. Material and methods

Plant material and setting up- The experiments were conducted in two commercial vineyards using *Vitis vinifera* L. cv Carménère and Syrah. During the 2017 growing season, the experiment was carried out in Viñedos Emiliana at Región del Libertador Bernardo O´Higgins, Chile with Carménère (massal selection, own-rooted) and Syrah (clone 100 grafted on Kober 5 BB rootstock, *V. berlandieri* x *V. riparia*). In 2018, the experiment was conducted in Haras de Pirque, at Región Metropolitana, Chile on Carménère (masal selection, own-rooted) and Syrah (clone 174, own rooted). Both sites had similar plant densities, and rows were east-west oriented in 2017 and north-south in 2018. In the central row eight and ten plants were selected for 2017 and 2018, respectively. Each plant was properly marked and each one was considered as an individual. The irrigation line was removed from these three rows in the first week of January in both years.

Plant measurements -For leaf water potentials (Ψ) and gas exchange in the field, sun-exposed fully expanded mature leaves were selected from one shoot on each plant leaving the central leaf for recording gas exchange and Ψ_{md} and Ψ_{pd}. Leaf gas exchange was measured 2-4 minutes before sampling the Ψ_{Leaf}, by means of a portable gas analyser (CIRAS-2, PP Systems Co. Ltd., USA). For traits related to pressure-volume curves, stem hydraulic vulnerability curves, stomatal responses to abscisic acid and photosynthetic performance, whole shoots were sampled early in the morning, placed inside of three black plastic bags with abundant wet paper towels, sealed and transported to the laboratory for analysis. The pressure-volume curve analysis was performed before the beginning of the drought assays in both years and post-drought analysis was carried out only in 2018. Procedures were as in (Tyree and Hammel 1972). Also, vulnerability curves were performed using 27 and 35 shoots from different irrigated Carménère and Syrah plants, respectively, during March of 2018, as in (Hochberg et al. 2016) and (Torres-Ruiz et al. 2015). Stomatal response to Abscisic acid (ABA) was assessed in detached leaves, by supplying ABA through the transpiration stream under illumination with ABA concentration of 25, 50, 100 and 150 µM. Finally, the effect of drought on photosynthetic performance was measured in detached leaves collected from the plants after resuming irrigation during March of 2018, illuminating the leaves with 800 µmol photons m^{-2} s^{-1} for 1 h, and assessing A-Ci responses as in (Duursma 2015).

1.1. Data analysis

Data analysis were performed using R software (version 3.3.3, R core team 2017), using available packages (Pinheiro and Bates 2000), (Lenth 2016), (Duursma 2014). Pressure volume results were compared using ANOVA and post hoc Tukey HSD test ($P<0.05$).

3. Results and discussion

3.1. *According to their σ value Syrah has a higher degree of isohydry than Carmenere*

Grapevine varieties have been classified along the isohydric to anisohydric continuum, according to the strategy to manage their water potential regulation. Considering that the extent of the isohydry is referred to the Ψ_{Leaf} responses to changes in soil water availability (Martínez-Vilalta and Garcia-Forner 2016), our results indicate that Carménère behaves as anisohydric ($\sigma \geq 1$) while in Syrah, despite differences between seasons, the $\sigma \leq 1$ in both sites (Table 1). Also, the Ψ_{leaf} to Ψ_{PD} relationship resulted in a strong linear fit indicating a constant σ, irrespective of season, along the Ψ_{pd} range usual to viticultural practices (Data not shown).

3.2. *Stomatal sensitivity to drought*

Regarding the stomatal sensitivity to drought, which corresponds to the slope of the g_s to Ψ_{stem} regression at 50% of the maximal g_s, was lower in Syrah 2017 compared to both, Carménère and Syrah 2018 (Table 1). Similarly, the Ψ_{gs12} value, which corresponds to the Ψ resulting in a 12% of the maximal g_s, in Syrah was significantly more negative during the 2017 seasons compared to the 2018 season and to Carménère in 2017 (Table 1).

3.3. *Isohydry degree, physiological responses to drought and drought resistance*

Independent on the degree of isohydry of the grapevine varieties, it is not clear what the consequences would be regarding the resistance to drought, even though some suggests that a near-anisohydric response would imply a higher drought tolerance than an isohydric behaviour (Tardieu and Simonneau 1998, Schultz 2003, McDowell et al. 2008, Sade et al. 2012, Skelton et al. 2015). If that would be the case it would be expected that plants with a higher degree of anisohydry would reach more negative Ψ thresholds necessary for physiological changes related to drought resistance such as Ψ_{gs12}, π_0, Ψ_{TLP} and Ψ_{PLC50}. On the contrary, Carménère, with a higher σ than Syrah, resulted in more positive values for Ψ_{gs12}, π_0 and Ψ_{TLP} (Table 1) compared to Syrah in 2017, even though with no differences in stomatal regulation compared to Syrah 2018 (Table 1). But also, when assessing the percent loss of conductivity in stems under shelf dehydration (PLC_{50} and PLC_{12}), Syrah was less prone to cavitation than Carménère (Table 1), and from our results, the Ψ_{Leaf} regulation does not depend on stomata (Table 1), similar to previous reports (Martínez-Vilalta and Garcia-Forner 2016). We suggest that the Ψ_{Leaf} regulation upon water availability is not a trait robust enough as to make straightforward inferences in relation to drought resistance in grapevines.

3.4. *Stomatal responses and photosynthetic performance*

Stomatal closure is mostly explained by turgor loss during dehydration under mild water stress conditions (Rodriguez-Dominguez et al. 2016), emerging the hypothesis that hydraulic signals close the stomata while ABA is important for sustaining a low gs under lower water potentials (Tombesi et al. 2015, Degu et al. 2019) or higher VPD (Speirs et al. 2013, Brodribb and McAdam 2015). The ABA fed through the transpiration stream in leaves, reduced the g_s down to 80% in both varieties, but in Syrah, the K_m for gs reduction by ABA was double than in Carménère, being the latter more sensitive to the hormone (Figure 1). Stomatal clousure, on the other hand, leads to strong reductions on the CO_2 concentration at the carboxylation sites, frequently leading to non-stomatal limitations to photosynthesis. From our results, and regardless of the variety, the more negative the Ψ_{min} experienced by leaves, the lower the Vc_{max} and the J_{max} after the recovery from water stress (Figure 2). Also, water stress is known to reduce electron transport in the photosynthetic apparatus by damage to the Mn-cluster at the PSII electron donor side, as well as both photosystem reaction centers (Toivonen and Vidaver 1988, He et al. 1995). From our results, the non-extreme water stress conditions, usual in grapevine production for wine making, might have a non-reversible impact on plants photosynthesis.

4. Conclusions

According to the Ψ_{PD} to Ψ_{Leaf} ratio, Syrah resulted in a higher degree of isohydry compared to Carménère. Also, the physiological responses of Carmenere, including Ψ_{gs12}, π_0, Ψ_{TLP} and Ψ_{PLC50}, despite having a higher isohydry degree, is less resistant to drought. Finally, stomatal sensitivity to ABA is higher in Carénère and, the drought effect on the photosynthetic performance is similar between varieties, irrespective of their degree of isohydry.

5. Acknowledgments

The authors thank the funding from CONICYT (FONDECYT Project Nº 1140880), also, the CONICYT + PAI, Concurso nacional tesis de doctorado en el sector productivo, convocatoria 2016 + Folio T7816120001, and the VID short visit funding support from the Universidad de Chile. We thank the Doctorado en Ciencias Silvoagropecuarias y Veterinarias (DCSAV) from the Universidad de Chile. We are grateful to Viñedos Emiliana for the plant material and the field support given, particularly to Andrés Aparicio and the Department of I+D+i. Also, we thank Haras de Pirque, and Julio Muñoz for their support and collaboration.

6. Litterature cited

Brodribb TJ, McAdam SAM (2013) Abscisic acid mediates a divergence in the drought response of two conifers. Plant Physiol 162:1370–7.

Bucci SJ, Goldstein G, Meinzer FC, Franco AC, Campanello P, Scholz FG (2004) Mechanisms contributing to seasonal homeostasis of minimum leaf water potential and predawn disequilibrium between soil and plant water potential in Neotropical savanna trees. Trees 19:296–304.

Charrier G, Delzon S, Domec J-C, Zhang L, Delmas CEL, Merlin I, Corso D, King A, Ojeda H, Ollat N, Prieto JA, Scholach T, Skinner P, van Leeuwen C, Gambetta GA (2018) Drought will not leave your glass empty: Low risk of hydraulic failure revealed by long-term drought observations in world's top wine regions. Sci Adv 4:eaao6969.

Duursma RA (2015) Plantecophys - An R Package for Analysing and Modelling Leaf Gas Exchange Data Struik PC (ed). PLoS One 10:e0143346.

He JX, Wang J, Liang HG (1995) Effects of Water-Stress on Photochemical Function and Protein-Metabolism of Photosystem-Ii in Wheat Leaves. Physiol Plant 93:771–777.

Hochberg U, Degu A, Fait A, Rachmilevitch S (2013) Near isohydric grapevine cultivar displays higher photosynthetic efficiency and photorespiration rates under drought stress as compared with near anisohydric grapevine cultivar. Physiol Plant 147:443–52.

Hochberg U, Herrera JC, Cochard H, Badel E (2016) Short-time xylem relaxation results in reliable quantification of embolism in grapevine petioles and sheds new light on their hydraulic strategy Meinzer F (ed). Tree Physiol 36:748–755.

Lenth R (2016) Least-squares means: the R package lsmeans. J Stat Softw.

Lovisolo C, Perrone I, Carra A, Ferrandino A, Flexas J, Medrano H, Schubert A (2010) Drought-induced changes in development and function of grapevine (Vitis spp.) organs and in their hydraulic and non-hydraulic interactions at the whole-plant level: A physiological and molecular update. Funct Plant Biol 37:98–116.

Martínez-Vilalta J, Garcia-Forner N (2016) Water potential regulation, stomatal behaviour and hydraulic transport under drought: deconstructing the iso/anisohydric concept. Plant Cell Environ:1–15.

Martínez-Vilalta J, Poyatos R, Aguadé D, Retana J, Mencuccini M (2014) A new look at water transport regulation in plants. New Phytol 204:105–115.

McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG, Yepez E (2008) Mechanisms of plant survival and mortality during drought: Why do some plants survive while others succumb to drought? New Phytol 178:719–739.

Pinheiro JC, Bates DM (2000) Mixed-effects models in S and S-PLUS. Springer.

Sade N, Gebremedhin A, Moshelion M (2012) Risk-taking plants: anisohydric behavior as a stress-resistance trait. Plant Signal Behav 7:767–70.

Schultz HR (2003) Differences in hydraulic architecture account for near-isohydric and anisohydric behaviour of two field-grown Vitis vinifera L. cultivars during drought. Plant, Cell Environ 26:1393–1405.

Skelton RP, West AG, Dawson TE (2015) Predicting plant vulnerability to drought in biodiverse regions using functional traits. Proc Natl Acad Sci U S A 112:5744–9.

Steudle E (2000) Water uptake by roots: effects of water deficit. J Exp Bot 51:1531–1542.

Tardieu F, Simonneau T (1998) Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours. J Exp Bot 49:419–432.

Tardieu F, Simonneau T, Parent B (2015) Modelling the coordination of the controls of stomatal aperture, transpiration, leaf growth, and abscisic acid: update and extension of the Tardieu-Davies model. J Exp Bot 66:2227–37.

Toivonen P, Vidaver W (1988) Variable Chlorophyll a Fluorescence and CO2 Uptake in Water-Stressed White Spruce Seedlings. Plant Physiol 86:744–748.

Tombesi S, Nardini A, Farinelli D, Palliotti A (2014) Relationships between stomatal behavior, xylem vulnerability to cavitation and leaf water relations in two cultivars of Vitis vinifera. Physiol Plant 152:453–464.

Torres-Ruiz JM, Jansen S, Choat B, McElrone AJ, Cochard H, Brodribb TJ, Badel E, Burlett R, Bouche PS, Brodersen CR, Li S, Morris H, Delzon S (2015) Direct x-ray microtomography observation confirms the induction of embolism upon xylem cutting under tension. Plant Physiol 167:40–3.

Table 1. Summary of hydraulic traits in grapevines of Carménère and Syrah during season 2017 and 2018. ⍰ describe the slope between midday leaf water potential (Ψ_{leaf}) and soil water potential (Ψ_{pd}), gs 50 and gs 12 to the Ψ_{stem} at 50% and 12% of the stomatal closure occur, π_0 and Ψ_{TLP} are the osmotic potential at full turgor and the Ψ at turgor loss point, respectively, and Ψ_{PLC12} and Ψ_{PLC50} to the Ψ at 12% and 50% loss of hydraulic conductance.

Hydraulic trait		***Carménère*** 2017		2018		***Syrah*** 2017		2018	
Isohydry degree									
⍰	(MPa MPa^{-1})	1,15	*a*	1,08	*a*	0,92	*a*	0,44	*b*
Stomatal sensitivity to⍰⍰									
gs 50	(MPa)	-1,09	*a*			-1,31	*b*	-1,06	*a*
gs 12	(MPa)	-1,33	*a*			-1,67	*b*	-1,23	*a*
Pressure-volume									
⍰$_0$	(MPa)	-1,36	*c*	-1,28	*b*	-1,64	*d*	-1,19	*a*
⍰$_{TLP}$	(MPa)	-1,80	*b*	-1,67	*b*	-2,00	*c*	-1,50	*a*
Shoot PLC									
⍰$_{PLC12}$	(MPa)			-0,74	*a*			-1,34	*b*
⍰$_{PLC50}$	(MPa)			-2,09	*a*			-2,62	*b*

Different letters indicate significant differences for the hydraulic traits observed between grapevines ($p < 0.05$).

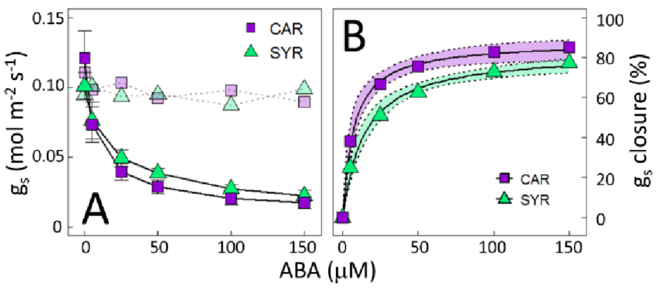

Figure 1. Stomatal response to increasing doses of ABA in grapevine leaves. (A) Average stomatal conductance when individual leaves of Carménère (squares) and Syrah (triangles) were fed with increasing doses of 0, 5, 25, 50, 100 and 150 µM of ABA for 50 min each concentration. Straight line represents the progression response of gs to increasing ABA doses and segmented line shows gs at equal period, but in leaves fed with water as control. (B) Percentage of stomatal conductance reduction (gclosure) from the initial value from each leaf. The gclosure response to ABA was fitted using Michaelis–Menten curve. For each curve, the predicted mean (black solid line) and the 99 % CI (segmented lines) are shown.

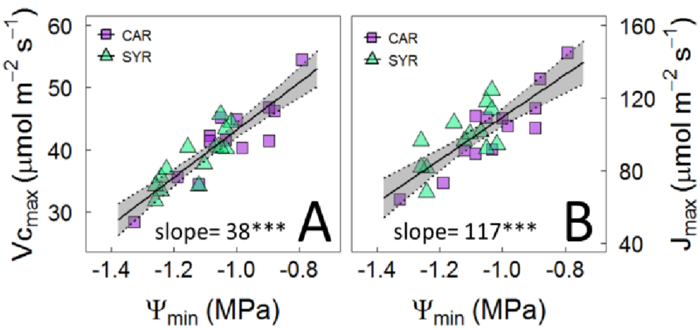

Figure X. Post-drought effects on the photosynthetic performance to the lowest values of ⍰$_{stem}$ (⍰$_{min}$) registered for each plant over the season 2018. (A) Response of Maximum rate of carboxylation and (B) Maximum rate of electron transport at photosynthetically active at radiation of 1000 µmol m^{-2} s^{-1}. For each curve, the predicted mean (black solid line) and the 99 % CI (segmented lines) are shown. Asterisks denotes significant slope, different from 0, *, P< 0.05, **, P<0.01; ***, P< 0.001.