

Responses of leaf stomatal density and anatomy to water deficit in four winegrape cultivars (*Vitis vinifera* L.)

Theodorou N., Koundouras S., Zioziou E., Nikolaou N.

Laboratory of Viticulture, School of Agriculture, Aristotle University of Thessaloniki, 541 24, Thessaloniki, Greece

Abstract

Grapevine performance under semi-arid conditions largely depends on acclimation to water limitation. *Vitis vinifera* genotypes have been reported to rely on different mechanisms of drought avoidance and tolerance mediated by both stomatal and non stomatal responses. However, although water relations under drought have been extensively studied in a variety of grapevine genotypes and conditions, changes in anatomical traits have been less frequently explored. The aim of this work was to investigate the effects of water deficit conditions on leaf stomata density and anatomy in four *Vitis vinifera* L. genotypes (Syrah, Grenache, Xinomavro and Agiorgitiko) during the 2012 season. Vines were subjected to three irrigation regimes [full irrigation (FI): 100% of crop evapotranspiration (ET_c), deficit irrigation (DI): 50% ET_c and non-irrigated (NI)] replicated in randomized blocks. Vine water relations were assessed by measurements of water potential and gas exchange parameters. In order to determine stomatal densities and dimensions, leaf epidermal imprints were collected from the underside of 9 mature leaves per treatment between the 7th and 9th node zone in which leaf gas exchange parameters were measured. Stomatal counts were conducted for 6 sub-fields of view for each imprint under an optical microscope equipped with an ocular cubic micrometer. All sections were photographed with a digital camera and the thickness of the palisade and spongy mesophyll was recorded. Water status and related physiological parameters differed significantly among irrigation treatments with lower levels in the non irrigated vines. Water deficit reduced water potential values but increased intrinsic water use efficiency (WUE). Progressive drought increased stomatal density and decreased stomatal size in Grenache and Xinomavro while the opposite was observed in Syrah and Agiorgitiko. The thickness of the palisade and spongy mesophyll increased with water deficit in

Grenache and Xinomavro but decreased in Syrah and Agiorgitiko. The results indicate differences in anatomical responses to drought among grapevine genotypes which could be possibly associated with different adaptation strategies to water limitation.

Key words: stomatal density, water deficit, leaf anatomical traits, leaf thickness.

Introduction

Grapevine is a worldwide spread crop, mainly in regions where a Mediterranean climate type occurs (Schultz and Stoll, 2009). In such climates, rainfall is unevenly distributed throughout the year, resulting in an elevated evaporative demand during summer and affecting critical vine characteristics such as vigour, canopy size and berry growth and composition. Thus, improving water use efficiency (WUE) is an important issue in situations of intense summer drought.

Stomatal behaviour and characteristics, together with leaf morphological and anatomical traits may play a key role in plants responses to water deficit. Although these mechanisms are under tight genetic control (Boso et al., 2011), they can be altered in response to environmental conditions, thereby contributing in plant adaptation mechanisms to drought by minimizing water loss and enhancing WUE. Stomatal density and dimensions in plants have been reported to change with drought, vegetative growth, elevation, wind (Gokbayrak et al., 2008), air CO₂ levels (Rogiers et al., 2011) and salt stress (Zhao et al., 2006).

Physiological and biochemical responses of grapevines to drought have been investigated in previous studies. However, the association between physiological and morpho-anatomical adaptations of grapevine leaves under water stress conditions is not yet fully understood. The aim of this study was (a) to examine the modifications that occur at the leaf and stomata level under drought conditions and (b) to compare the adaptive responses of four red grapevine (*Vitis vinifera* L.) varieties to drought.

Materials and Methods

The study was carried out during the summer of 2012, in a 5-year-old vineyard planted in Thessaloniki, Greece, with four red grapevine (*Vitis vinifera* L.) varieties, respectively Grenache, Syrah, Xinomavro and Agiorgitiko. The experiment was

arranged as a 3 x 3 factorial design with tree irrigation regimes [full irrigation (FI), 100% of crop evapotranspiration (ET_c); deficit irrigation (DI), 50% ET_c; and non-irrigated (NI)]. Irrigation was scheduled on a weekly basis starting at berry set through harvest. The treatments were replicated 3 times in randomized blocks. In each plot, only the central four vines were used for measurements.

Vine water status was estimated by measurements of predawn (Ψ_{dawn}) and stem (Ψ_{stem}) water potentials using a pressure chamber. Measurement of Ψ_{dawn} started 1 hour prior to dawn while Ψ_{stem} was performed at solar noon (12 h to 13 h 30) on cloudless days. For Ψ_{stem} , three leaves of the inside part of the canopy were enclosed in plastic bags and covered with aluminium foil for at least 90 min before measurement to allow equilibration, according to the methodology of Choné et al. (2001).

Net assimilation rate (A) and stomatal conductance (g_s), were recorded at midday, simultaneously with Ψ_{stem} measurements, using the LCi portable gas exchange system (ADC BioScientific Ltd, Hoddesdon, UK). Measurements were taken on three fully expanded, recently matured, sun-light leaves per plot (photosynthetic photon flux density > 1200 $\mu\text{mol m}^{-2}\text{s}^{-1}$) and adjacent to those used for Ψ_s determination. Intrinsic WUE was calculated as the A/ g_s ratio. Gas exchange measurements were conducted at midday in order to obtain an accurate indication of grapevine response to environmental stress (Medrano et al., 2003).

Nine mature and fully developed leaves per treatment from the 7th to the 9th node from the base (O.I.V, 2009; Boso et al., 2011) were sampled at harvest. Abaxial epidermis imprints were taken using clear nail polish from 3 different parts of leaf lamina per leaf. Imprints were mounted on a glass slide with a cover slide (Xu and Zhou, 2008) and were observed under a Light microscope (Carl Zeiss 62786, Germany) equipped with a digital photo camera (Canon PowerShot, A630). For each imprint, number of stomata/ mm^2 (average number of 3 fields) and stomata dimensions (10 stomata randomly selected per field) were measured. For the anatomical study, transversal sections of leaf lamina were taken from both sides of the midvein and observed under the microscope. Measurements of stomatal dimensions and leaf anatomical traits were conducted using the Image J v1, 46R software (public domain Java image processing and analysis program, USA).

Results and Discussion

Ψ_{stem} and Ψ_{dawn} were significantly different between irrigation treatments in all varieties, with lower levels in NI treatments (Table 1). Mean values for NI ($\Psi_{\text{stem}} = -1,52$ to $-2,01$ and $\Psi_{\text{dawn}} = -0,81$ to $-1,03$) corresponded to a moderate to severe water limitation (van Leeuwen et al., 2009). Fully irrigated vines were subjected to a mild water limitation while DI plants had intermediate values in all varieties. Net photosynthesis (A) and stomatal conductance (g_s) mean values followed the same pattern as Ψ_{stem} and Ψ_{dawn} , with decreasing values from FI to NI for all varieties. Water deficit conditions may lead to a depression of photosynthetic rate due to stomatal and non stomatal limitations (Escalona et al., 1999). It is generally assumed that drought-induced decreases of photosynthesis are primarily attributed to stomatal closure, limiting CO₂ availability in the mesophyll. However, there is strong evidence that severe and prolonged water stress can affect mesophyll metabolism and reduce photosynthetic capacity as a consequence of decreased ribulose biphosphate regeneration (Giménez et al. 1992). However, in the conditions of this experiment, intrinsic WUE was lower under full irrigation and maximized in NI plants, suggesting that, on average during the season, severe water stress was avoided and stomatal limitations were the main cause for the reduction in photosynthesis.

Table 1. Water status and gas exchange parameters mean values for the 2012 season, in the four varieties (n=9). FI: full irrigated; DI: deficit irrigated; NI: non irrigated.

		Ψ_{dawn} (Mpa)	Ψ_{stem} (Mpa)	A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	g_s ($\text{mol m}^{-2} \text{s}^{-1}$)	WUE ($\mu\text{mol mol}^{-1}$)
Grenache	FI	-0.33 a	-0.68 a	21.3 a	0.58 a	40.7 c
	DI	-0.53 b	-1.21 b	15.5 b	0.33 b	51.9 b
	NI	-1.03 c	-2.01 c	3.1 c	0.04 c	73.0 a
Syrah	FI	-0.37 a	-0.76 a	20.9 a	0.62 a	36.2 c
	DI	-0.53 b	-1.19 b	15.0 b	0.29 b	54.2 b
	NI	-0.88 c	-1.75 c	5.9 c	0.09 c	66.3 a
Xinomavro	FI	-0.42 a	-0.74 a	19.9 a	0.47 a	44.4 b
	DI	-0.59 b	-1.08 b	15.8 b	0.37 b	45.9 b
	NI	-0.81 c	-1.52 c	7.8 c	0.15 c	56.1 a
Agiorgitiko	FI	-0.35 a	-0.71 a	19.4 a	0.55 a	38.9 b
	DI	-0.59 b	-1.18 b	11.5 b	0.20 b	60.1 a
	NI	-0.86 c	-1.56 c	2.8 c	0.05 c	60.2 a

Means with different letters within a variety are significantly different ($P < 0.05$).

Observations of the abaxial epidermis showed that there were significant changes of stomatal density and dimensions between irrigation treatments, especially in Xinomavro, Grenache and Agiorgitiko (Table 2). There was an increase in stomatal density with increasing drought in Xinomavro and Grenache leaves, followed by a decrease in stomatal length and width from FI to NI. These differences could be attributed to a possible adaptation mechanism for limiting water losses through a better control of transpiration. Indeed, previous studies (Bosabalidis and Kofidis, 2002; Xu and Zhou, 2008; Laajimi et al., 2011) reported an increase of stomatal density under water limitation conditions coupled by a decrease in stomatal size in olive and apricot trees. Small guard cells of leaf stomata may also contribute to balance CO₂ uptake and water loss through stomatal pores while maintaining g_s values to favorable levels (Xu and Zhou, 2008). In contrast, Agiorgitiko and Syrah showed a different response, as stomatal density decreased and stomata length and width increased from FI to NI (except for stomata width in Agiorgitiko). Zhang et al. (2006) reported higher stomata length and lower width under water stress conditions in wheat plants.

Table 2. Stomatal density, length and width at harvest of the 2012 season, in the four varieties (n=9). FI: full irrigated; DI: deficit irrigated; NI: non irrigated.

		Stomatal density (No/mm ²)	Stomata Legth (mm)	Stomata Width (mm)
Grenache	FI	200 ± 5 b	34.3 ± 0.7 a	20.5 ± 0.2 a
	DI	240 ± 9 a	28.7 ± 0.3 b	18.3 ± 0.1 b
	NI	221 ± 6 a	28.6 ± 0.6 b	18.2 ± 0.2 b
Syrah	FI	228 ± 8	28.0 ± 0.4 b	20.1 ± 0.5 b
	DI	216 ± 4	29.3 ± 0.6 ab	21.0 ± 0.2 b
	NI	215 ± 9	30.6 ± 0.6 a	22.0 ± 0.6 a
Xinomavro	FI	225 ± 9 b	29.5 ± 0.6 a	21.2 ± 0.1 a
	DI	258 ± 3 a	28.3 ± 0.2 a	19.5 ± 0.3 b
	NI	280 ± 9 a	26.2 ± 0.5 b	18.4 ± 0.2 c
Agiorgitiko	FI	249 ± 4 a	28.6 ± 0.2 b	20.1 ± 0.3 a
	DI	226 ± 7 b	28.0 ± 0.5 b	18.7 ± 0.2 b
	NI	218 ± 4 b	31.3 ± 0.2 a	20.5 ± 0.2 a

Means with different letters within a variety are significantly different (P < 0.05).

Observation of cross-sections of leaves showed that there were significant changes in leaf anatomical characteristics induced by water stress. In particular, water stress

resulted in a significant decrease of the thickness of spongy parenchyma in Syrah, as well as of the entire lamina thickness (Table 3). Similarly to these results, total thickness of leaves of water-stressed plants was lower than control plants in avocado (Chartzoulakis et al., 2002) and apricot trees (Laajimi et al., 2011). On the contrary, in Grenache and Agiorgitiko, water restriction resulted in an increase of the thickness of almost all histological components of the mesophyll, as well as of the entire lamina thickness. In the latter varieties, the increase of the thickness of palisade parenchyma could lead to a higher number of CO₂ assimilation sites and thus contribute to maintain high A values at low g_s. Increase of total lamina thickness under water stress conditions was reported previously for *Olea europaea* (Ennajeh et al., 2010) and was considered as a key structural adaptation process, responsible in part for the observed differences in drought resistance among varieties. Xinomavro showed no significant changes of anatomical traits except for an increased spongy parenchyma thickness in NI.

Table 3. Anatomical characteristics of leaf lamina cross sections, in μm , at 2012 harvest, in the four varieties (n=9). FI: full irrigated; DI: deficit irrigated; NI: non irrigated.

		Total	Upper epidermis	Palisade parenchyma	Spongy parenchyma	Lower epidermis
Xinomavro	FI	185.3	11.07	58.62 b	104.85	10.54
	DI	185.5	11.35	61.88 ab	101.62	10.69
	NI	190.0	11.37	67.69 a	99.77	11.51
Grenache	FI	215.3 b	15.65	67.97 c	117.41	14.30
	DI	216.3 b	14.71	74.88 b	112.35	14.42
	NI	228.1 a	14.81	75.78 a	123.45	14.06
Syrah	FI	196.4 a	10.87	68.14	106.43 a	11.02
	DI	195.3 a	11.66	65.72	105.93 a	11.99
	NI	182.9 b	9.57	65.75	97.53 b	10.15
Agiorgitiko	FI	208.8 b	10.35 c	71.10 b	116.11	11.27
	DI	213.5 b	11.75 b	72.04 b	116.65	13.12
	NI	215.4 a	12.02 a	75.73 a	114.54	12.13

Means with different letters within a variety are significantly different ($P < 0.05$).

Conclusions

Leaf morpho-anatomical adaptations to water deficit could participate alongside physiological ones in drought resistance in grapevine cultivars. In particular, drought-induced modifications in the thicknesses of the palisade and spongy parenchyma, stomatal density and size could be considered important adaptation mechanisms responsible, in part, for the observed intra-specific variability in the response of grapevines to water stress. However more studies are needed to confirm these results, in order to obtain a clearer picture of the multitude of drought resistance strategies in grapevines.

References

1. Bosabalidis A.M. and Kofidis G., 2002. Comparative effects of drought stress on leaf anatomy of two olive cultivars. *Plant Sci.* 163, 375-379.
2. Boso S.; Gago P.; Villaverde V.A.; Santiago J.L.; Mendez J.; Pazos I. and Martinez M.C., 2011. Variability at the electron microscopic level in leaves of members of the genus *Vitis*. *Scientia Hortic.* 128, 228-238.
3. Chartzoulakis K.; Patakas A.; Kofidis C.; Bosabalidis A. and Nastou A., 2002. Water stress affects leaf anatomy, gas exchange, water relations and growth of two avocado cultivars. *Scientia Hortic.* 95, 39–50.
4. Choné, X.; van Leeuwen, C.; Dubourdieu, D. and Gaudillère, J.P., 2001. Stem water potential is a sensitive indicator of grapevine water status. *Ann. Bot.* 87, 477–483
5. Ennajeh M.; Vadel A.M.; Cochard H. and Khemira H., 2010. Comparative impacts of water stress on the leaf anatomy of a drought-resistant and a drought-sensitive olive cultivar. *J. Hortic. Sci. Biotech.* 85, 289-294.
6. Escalona J. M.; Flexas J. and Medrano H., 1999. Stomatal and non-stomatal limitations of photosynthesis under water stress in field-grown grapevines. *Aust. J. Plant Physiol.*, 26, 421–433.
7. Gokbayrak Z.; Dardeniz A. and Bal M., 2008. Stomatal density of grapevine to windy conditions. *Trakia J. Sci.* 6, 18-22.
8. Giménez, C.; Mitchell, V.J. and Lawlor, D., 1992. Regulation of photosynthesis rate of two sunflower hybrids under water stress. *Plant Physiol.* 98, 516-524.

9. Laajimi N.O.; Boussadia O.; Skhiri F.H.; Teixeira da Silva J.A.; Rezgui S. and Hellali R., 2011. Anatomical adaptations in vegetative structures of Apricot tree (*Prunus armeniaca* L.) cv. 'Amor El Euch' grown under water stress. *Fruit, Vegetable and Cereal Sci. Biotech.* 5, 46-51.
10. Medrano, H.; Escalona, J.M.; Cifre, J.; Bota, J. and Flexas, J., 2003. A ten-year study on the physiology of two Spanish grapevine cultivars under field conditions: effects of water availability from leaf photosynthesis to grape yield and quality. *Funct. Plant Biol.* 30, 607–619.
11. Rogiers S.Y.; Hardie W.J. and Smith J.P., 2011. Stomatal density of grapevine leaves (*Vitis vinifera* L.) responds to soil temperature and atmospheric carbon dioxide. *Aust. J. Grape Wine Res.* 17, 147-152.
12. Schultz H.R. and Stoll M., 2010. Some critical issues in environmental physiology of grapevines: future challenges and current limitations. *Aust. J. Grape Wine Res.* 16, 4-24.
13. Van Leeuwen C.; Tregoat O.; Choné X.; Bois B.; Pernet D. and Gaudillère J.-P., 2009. Vine water status is a key factor in grape ripening and vintage quality for red Bordeaux wine. How can it be assessed for vineyard management purposes? *J. Int. Sci. Vigne Vin* 43, 121-134.
14. Xu Z. and Zhou G., 2008. Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. *J. of Exp. Bot.* 59, 3317-3325.
15. Zhang Y.P.; Wang Z.M.; Wu Y.C. and Zhang X. 2006. Stomatal characteristics of different green organs in wheat under different irrigation regimes. *Acta Agronomica Sinica* 32, 70-75.
16. Zhao S.; Chen W.; Ma D. and Zhao F. 2006. Influence of different salt level on stomatal character in rice leaves. *Reclaiming and Rice Cultivation* 6, 26-29.