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Technical Note

CHARACTERIZATION OF STOMATAL DENSITY AND SIZE OF DIFFERENT *VITIS VINIFERA* L. CULTIVARS GROWING IN MEDITERRANEAN CLIMATE CONDITIONS

CARACTERIZAÇÃO DA DENSIDADE E TAMANHO DOS ESTOMAS DE DIFERENTES CULTIVARES DE *VITIS VINIFERA* L. CULTIVADAS EM CONDIÇÕES DE CLIMA MEDITERRÂNEO

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SUMMARY

The stomatal traits of 13 red and white grapevine cultivars of *Vitis vinifera* L. established in a cultivar collection garden in central Chile were characterized in this study. The cultivars examined were 'Cabernet Franc', 'Cabernet Sauvignon', 'Carmenere', 'Cot', 'Grenache', 'Merlot', 'Mourvedre', 'Pinot Noir', 'Syrah', 'Chardonnay', 'Marzanne', 'Roussane', and 'Sauvignon Blanc'. Transparent nail polish peel prints in four adult leaves per vine were obtained and examined under a light microscope at 10 ×. The number of stomata in 0.196 mm^2 was counted, and their length and width were measured using image analysis software. With these variables, the stomatal area and stomatal density were obtained. The 'Cot' cultivar had the largest stomata and stomatal area, while the 'Mourvedre' cultivar had the smallest stomata and stomatal area. 'Chardonnay' had the highest number of stomata and stomatal density, while 'Carmenere' had the lowest. The red grapevine cultivars exhibited larger and fewer stomata than the white grapevine cultivars, corroborating different adaptations for the environmental modulation of stomatal conductance and transpiration.

RESUMO

Neste estudo foram avaliadas diferentes características estomáticas em 13 cultivares de videira tintas e brancas de *Vitis vinifera* L. plantadas num jardim de castas no centro do Chile. As cultivares examinadas foram 'Cabernet Franc', 'Cabernet Sauvignon', 'Carmenere', 'Cot', 'Grenache', 'Merlot', 'Mourvedre', 'Pinot Noir', 'Syrah', 'Chardonnay', 'Marzanne', 'Roussane' e 'Sauvignon Blanc'. Impressões transparentes de casca de esmalte em quatro folhas adultas por videira foram obtidas e examinadas em microscópio ótico a 10 ×. O número de estomas em 0,196 mm^2 foi contado e seu comprimento e largura foram medidos por software de análise de imagem. Com essas variáveis, foram obtidas a área estomática e a densidade estomática. A cultivar 'Cot' apresentou os maiores estomas e a maior área estomática, enquanto a cultivar 'Mourvedre' apresentou resultados opostos. 'Chardonnay' e 'Carmenere' foram as cultivares com maior e menor número de estomas e densidade estomática. As cultivares de videiras tintas apresentaram estomas maiores e em menor número que as cultivares de videiras brancas, corroborando diferentes adaptações para a modulação ambiental da condutância estomática e transpiração.

Keywords: Stomatal morphology, grapevine cultivars, environmental modulation.
Palavras-chave: Morfologia dos estomas, variedades de videira, modulação ambiental.

INTRODUCTION

Stomatal pores are microscopic openings on the leaf epidermis that enables plants to regulate water use and carbon gain. Stomatal traits such as length, width, and the number of stomata vary among plant species, varieties, and even clones (Sophie *et al.*, 2008). Environmental carbon dioxide concentration and temperature also play an essential role in the changes in stomatal morphology, which can then affect water use efficiency in plants (Lawson and Blatt, 2014; Lawson and Vialet-Chabrand, 2019). The density and size of stomata are involved in the environmental modulation of stomatal conductance and are invariant after the leaf is fully expanded (Rogiers *et al.*, 2009). The size and number of stomata are considered adaptations to control transpiration in plants, and small stomata are considered an adaptation to minimize transpiration (Jones, 2014). Thus, knowledge of phenotypic variation in stomatal morphology can support the development of genotypes able to sustain yields in environments with less water.

Grapevine (*Vitis vinifera* L.) is a widely cultivated crop that can be grown in a wide range of climates, from oceanic to arid and hyperarid (Tonietto and Carbonneau, 2004; Schultz and Stoll, 2010). In studies on the vine stomatal morphology, Boso *et al.* (2016) reported significant phenotypic variation in stomatal traits of 12 varieties of *V. vinifera*. Stomatal length and width varied from 35 to 48 μm and from 21 to 30 μm in varieties 'Treixadura' and DHP 'Jacquez', respectively. Similarly, Bodor *et al.* (2019) showed that stomatal anatomy varied within the canopy of *V. vinifera* and the number of stomata increased from the 1st to the 10th node of the shoot. In Chile, the total area cultivated with *V. vinifera*, in the Mediterranean-type climate zones is *ca.* 145,000 ha (ODEPA, 2022); however, no studies have been conducted to describe stomatal morphology among cultivars of *V. vinifera*, which could help to understand the adaptive mechanisms of the species to face water stress. This study aimed to characterize the stomatal density and size for different *V. vinifera* cultivars grown in a cultivar collection garden in central Chile. The relationship between stomatal morphology and grape color (white *versus* red cultivars) was also addressed.

MATERIALS AND METHODS

The plant material was obtained from a cultivar collection garden at the Universidad de Talca, located in the Panguillemo Valley, Maule region of Chile (35°24S and 71°37W, 150 m above sea level). The vineyard was managed using optimal agronomic practices, including irrigation, weed control, and fertilization. Thirteen cultivars of *V. vinifera* ('Cabernet franc', 'Cabernet Sauvignon', 'Carmenere', 'Cot', 'Grenache', 'Merlot', 'Mourvedre', 'Pinot Noir', 'Syrah', 'Chardonnay', 'Marzanne', 'Roussane', and 'Sauvignon Blanc') were used in this study. These cultivars were planted in 2006, irrigated with a drip irrigation system, and trained using a Vertical Shoot Position system. The study area has a dry Mediterranean climate with an average annual precipitation of 600 mm, mainly concentrated in the autumn-winter period (De la Fuente *et al.*, 2018). The mean annual precipitation, temperature, and radiation for the 2012-2013 growing season were 286 mm, 13.9 °C, and 17.8 Mj/m^2/d, respectively. During the 2012-2013 growing season, two vines per cultivar were sampled by collecting four fully expanded, mature (6 to 8 months), sun-exposed, and healthy leaves of similar size from the 4th node of the shoot. Leaves were sampled at similar cumulative growing degree days from budburst, and stored at 4° C. Two nail polish peel prints were made in the lower epidermis of the middle of each leaf. A thin layer of nail varnish was applied and allowed to dry. Then, it was peeled off with a scalpel. Each sample was observed through a microscope slide with an objective of 10 ×, capturing three photographs per sample with a digital camera. The number of stomata (N) in 0.196 mm^2, length (SL, μm) and width (SW, μm) were counted and measured in MatLab software. The stomata area (SA, μm^2) and stomata density (SD, N per mm^2) were estimated using SL and SW. The ellipse formula was used to estimate SA. The length of the semi-major and semi-minor axes were obtained by dividing SL and SW by 2 (Equation 1).

$$SA = \pi * \frac{SL}{2} * \frac{SW}{2} \qquad \textbf{Eq. 1}$$

The analyses for each stomatal trait were carried out on a leaf-mean basis. The data were analyzed using one-way ANOVA with cultivar as the main factor. To meet the assumptions of normality and constant variances, values for stomatal traits were transformed using the Box-Cox transformation when the confidence intervals for the optimal λ did not include 1. Post-hoc mean comparisons for stomatal traits were based on the Tukey test. Orthogonal contrast analysis was used to test for differences between red grapevine cultivars ('Cabernet franc', 'Cabernet Sauvignon', 'Carmenere', 'Cot', 'Grenache', 'Merlot', 'Mourvedre', 'Pinot Noir', and 'Syrah') and white grapevine cultivars ('Chardonnay', 'Marzanne', 'Roussane', and 'Sauvignon Blanc'). Significant differences were obtained at $p<0.05$. All analyses were performed using SPSS version 22 (SPSS Inc, Chicago, Illinois, USA).

RESULTS AND DISCUSSION

The present study reveals significant differences in stomata anatomy among the 13 cultivars of *V. vinifera*, which supports the findings that stomata size and number vary significantly between different plant species (Bertolino *et al.*, 2019). Tukey tests revealed significant differences in all stomatal traits among cultivars. 'Cot' had the largest SL and SA, while 'Mourvedre' had the smallest. The cultivars with the highest N and lowest SD were 'Chardonnay' and 'Carmenere' (Table I). Similarly, the studies of Bodor *et al.* (2019), Teixeira *et al.* (2018), and Boso *et al.* (2016) found significant differences in the stomata morphology among different *V. vinifera* cultivars grown in Hungary, Portugal, and Spain, respectively. The authors found that the stomata were larger and wider (average SL and SW of 42 and 25 μm, respectively) than those observed in this study, but stomatal density was lower (30 stomata per mm^2). The orthogonal contrasts showed that all stomatal traits, except SW, differed between red and white grapevine cultivars. White grapevine cultivars had 22%, 22%, and 19% higher N, SA, and SD, respectively, than the red ones. However, SL was 4% lower in red grapevine cultivars (Table II).

Table I

Means and standard errors (in parentheses) for stomatal traits in the 13 cultivars of *V. vinifera* under study

Cultivar	Stomatal traits				
	SL (μm)	**SW (μm)**	**N**	**SD (N/mm^2)**	**SA (μm^2)**
Red					
'Cabernet Franc'	23.7 (0.16) cd	16.1 (0.18) bcde	32.2 (1.13) bc	135.4 (4.78) bc	301.0 (5.02) bcd
'Cabernet Sauvignon'	23.0 (0.28) de	15.3 (0.16) de	27.5 (1.09) cd	115.4 (4.61) cd	278.1 (5.95) def
'Carmenere'	25.0 (0.34) abc	16.4 (0.26) abcd	24.1 (1.11) d	101.5 (4.66) d	324.6 (9.30) abc
'Cot'	25.9 (0.28) a	17.3 (0.18) ab	31.1 (1.32) bc	130.9 (5.57) bc	352.7 (6.65) a
'Grenache'	25.9 (0.27) a	15.8 (0.27) cde	32.3 (0.98) bc	135.6 (4.14) bc	323.7 (7.41) abc
'Merlot'	25.1 (0.32) ab	16.4 (0.35) abcd	29.1 (1.44) cd	122.3 (6.04) cd	325.0 (10.66) abc
'Mourvedre'	21.9 (0.44) e	13.8 (0.31) f	26.8 (1.34) cd	112.5 (5.62) cd	238.7 (9.69) f
'Pinot Noir'	24.1 (0.25) bcd	17.6 (0.24) a	37.2 (1.59) ab	156.4 (6.70) ab	334.6 (7.44) ab
'Syrah'	23.5 (0.29) d	15.8 (0.27) cde	29.8 (1.23) cd	125.1 (5.18) cd	293.6 (8.42) cde
White					
'Chardonnay'	23.7 (0.16) bcd	16.2 (0.22) bcde	43.5 (1.70) a	182.9 (7.15) a	303.7 (5.49) bcd
'Marzanne'	22.0 (0.31) e	14.9 (0.34) ef	29.4 (1.69) cd	123.6 (7.10) cd	261.3 (9.13) ef
'Roussane'	23.5 (0.35) d	16.3 (0.36) abcd	42.3 (1.40) a	177.9 (5.90) a	304.6 (11.5) bcd
'Sauvignon Blanc'	24.3 (0.32) bcd	16.6 (0.25) abc	39.7 (0.80) a	166.9 (3.39) a	320.3 (8.28) abc

SL = Stomatal Length, SW = Stomatal Width, N = Number of stomata, SD = Stomatal Density, SA = Stomatal Area. Different letters within the same column indicate significant differences between cultivars ($p<0.05$).

Table II

Means, standard errors (in parentheses) and p-values for the estimated orthogonal contrast on stomatal traits between red- versus white grapevine cultivars

Trait	Type of grape		
	Red	White	p-value
SL (μm)	24.3 (0.13)	23.4 (0.18)	0.000
SW (μm)	16.1 (0.11)	16.0 (0.16)	0.992
N	30.1 (0.51)	38.7 (0.99)	0.000
SD (N/mm^2)	126.7 (2.15)	162.8 (4.18)	0.000
SA (μm^2)	311.0 (3.61)	297.5 (5.13)	0.035

SL = Stoma length, SW = stoma width, N = number of stomata per mm^2, SD = stoma density, SA = Stomata area.

Differences in stomatal morphology are associated with variations in light, air humidity, water availability, and atmospheric carbon dioxide concentration (Carson and Gray, 2008). The observed differences in stomatal traits between cultivars under study are likely due to genetic adaptations to the site conditions of their geographical origin, as the 13 cultivars of *V. vinifera* were grown under optimal agronomical conditions, which minimized environmental variations. Stomatal traits such as size and density influence plant photosynthesis, stomatal conductance, and water use efficiency (Franks and Beerling, 2009; Dow *et al.*, 2014). Plants with dense stomata generally have higher stomatal conductance and improved water use efficiency. In contrast, reductions in stomatal density can lead to conservative use of water because of decreased stomatal conductance and transpiration (Dittberner *et al.*, 2018). 'Chardonnay', an anysohidric white grapevine cultivar originated from a mesic environment in France, might be an example of this (Schultz, 2003; Chaves *et al.*, 2010). It has ~163 stomata per mm^2 and a high water use efficiency under water stress conditions (Gutiérrez-Gamboa *et al.*, 2019). Anisohydric cultivars of *V. vinifera* generally have a better performance in gas exchange parameters than isohydric cultivars under moderate water stress conditions (Pou *et al.*, 2012). However, under severe water stress conditions, anisohydric cultivars may suffer dehydration (Chaves *et al.*, 2016) due to their greater density of stomata, which implies a higher hydraulic conductance and vulnerability to embolism (Gerzon *et al.*, 2015). On the other hand, red grapevine cultivars had a lower stomatal density (~127 stomata per mm^2) which can drive to a conservative use of water because of the decrease in stomatal conductance and transpiration. For example, the isohydric cultivar 'Mourvedre', which is native to the Mediterranean region of Portugal, has lower stomatal size and density than other cultivars. This adaptation may help the plant conserve water under water stress conditions by minimizing transpiration (Poni *et al.*, 2007; Jones 2014; Serra *et al.*, 2017). Consequently, as stomata density is considered an evolutionary adaptation rather than a short-term acclimation mechanism, the results of the present study should be complemented with determinations of stomatal conductance and midday leaf water potential to support the adaptations and strategies of the different cultivars under drought conditions.

CONCLUSIONS

This study provides insights into the stomatal traits of red and white grapevine cultivars of *V. vinifera,* which help regulate acquisition and carbon gain, and corroborates different adaptations for the environmental modulation of stomatal conductance and transpiration. These findings could be used to select better-adapted genotypes for future warmer and dry conditions in Mediterranean-type climate areas.

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CONFLICTS OF INTEREST: The authors declare no conflict of interest.

REFERENCES

Bertolino L.T., Caine R.S., Gray J.E, 2019. Impact of stomatal density and morphology on water-use efficiency in a changing world. *Front. Plant Sci.*, **10,** 225.

Bodor P., Szekszárdi A., Varga Z., Bálo B., 2019. Investigation of the stomata size and frequency of grapevine (*Vitis vinifera* L.) cultivar 'Kékfrankos'. *Columella*, **6,** 29-34.

Boso S., Gago P., Alonso-Villaverde V., Santiago J.L., Martínez M.C., 2016. Density and size of stomata in the leaves of different hybrids (Vitis sp.) and *Vitis vinifera* varieties. *Vitis*, **55,** 17-22.

Carson S., Gray J.E., 2008. Influence of environmental factors on stomatal development. *New Phytol.*, **178,** 9-23.

Chaves, M.M., Costa, J.M., Zarrouk, O., Pinheiro, C., Lopes, C.M., Pereira, J.S., 2016. Controlling stomatal aperture in semi-arid regions – The dilemma of saving water or being cool? *Plant Sci.*, **251**, 54-64.

Chaves M.M., Zarrouk O., Francisco R., Costa J.M., Santos T., Regalado A.P., Rodrigues M.L., Lopes C.M., 2010. Grapevine under deficit irrigation: hints from physiological and molecular data. *Ann. Bot.*, **105,** 661–676, 2010.

De la Fuente-Pañitur C., Valdés-Gómez H., Roudet J., Acevedo-Opazo C., Verdugo-Vásquez N., Araya-Alman M., Lolas M., Moreno Y., Fermaund M., 2018. Classification of winegrape cultivars in Chile and France according to their susceptibility to *Botrytis cinerea* related to fruit maturity. *Aust. J. Grape Wine Res.*, **24,** 145–157.

Dittberner H., Korte A., Mettler-Altmann T., Weber A.P.M., Monroe G., de Meaux J., 2018. Natural variation in stomata size contributes to the local adaptation of water-use efficiency in *Arabidopsis thaliana*. *Mol. Ecol.*, **27,** 4052-4065.

Dow G.J., Bergmann D.C., 2014. Patterning and processes: how stomatal development defines physiological potential. *Curr. Opin. Plant Biol.*, **21,** 67–74.

Franks P.J., Beerling D.J., 2009. Maximum leaf conductance driven by CO2 effects on stomatal size and density over geologic time. *Proc. Natl. Acad. Sci.*, **106**, 10343-10347.

Gerzon E., Biton I., Yaniv Y., Zemach, H., Netzer, Y., Schwartz, A., Fait, A., Ben-Ari, G., 2015. Grapevine Anatomy as a Possible Determinant of Isohydric or Anisohydric Behavior. *Am. J. Enol. Vitic.*, **66**, 340-347.

Gutiérrez-Gamboa G., Pérez-Donoso A.G., Pou-Mir A., Acevedo-Opazo C., Valdés-Gómez H., 2019. Hydric behaviour and gas exchange in different grapevine varieties (*Vitis vinifera* L.) from the Maule Valley (Chile). *S. Afr. J. Enol. Vitic.*, **40**, 1-1.

Jones H.G., 2014. Drought and other abiotic stresses. *In: Plants and microclimate: A quantitative approach to environmental plant physiology*. 255-289. Jones H.G. (ed.), Cambridge University Press, Cambridge.

Lawson T., Blatt M.R., 2014. Stomatal size, speed, and responsiveness impact on photosynthesis and water use efficiency. *Plant Physiol.*, **164,** 1556–1570.

Lawson T., Vialet-Chabrand S., 2019. Speedy stomata, photosynthesis and plant water use efficiency. *New Phytol.*, **221,** 93–98.

ODEPA - Oficina de Estudios y Políticas Agrarias., 2022. Catastro vitícola nacional. Available at: https://www.odepa.gob.cl/rubro/vinos/catastro-viticola-nacional (accessed on 22.05.2023).

Poni S., Bernizzoni F., Civardi S., 2007. Response of Sangiovese grapevines to partial rootzone drying: gas-exchange, growth and grape composition. *Sci. Hortic.*, **114,** 96–103.

Pou A., Flexas J., Alsina M.M., Bota J., Carambula C., de Herralde F., Galmés J., Lovisolo C., Jiménez M., Ribas-Carbo M., Rusjan D., Secchi F., Tomàs M., Zsófi Z,, Medrano H., 2008. Adjustments of water-use efficiency by stomatal regulation during drought and recovery in the drought-adapted Vitis hybrid Richter-110 (*V. berlandieri* x *V. rupestris*). *Physiol. Plantar.*, **134**, 313–323.

Rogiers S.Y., Greer D.H., Hutton R.J., Landsberg J.J., 2009. Does night-time transpiration contribute to anisohydric behaviour in a *Vitis vinifera* cultivar? *J. Exp. Bot.*, **60,** 3751–3763.

Schultz H.R., 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.

Schultz H.R., Stoll M., 2010. Some critical issues in environmental physiology of grapevines: future challenges and current limitations. *Aust. J. Grape Wine Res.*, **16,** 4-24.

Serra I., Strever A., Myburgh P., Schmeisser M., Deloire P.A., 2017. Grapevine (*Vitis vinifera* L. 'Pinotage') leaf stomatal size and density as modulated by different rootstocks and scion water status. *Acta Hortic.*, **157,** 177-181.

Sophie Y.D., Marron N., Barbra K., Reinhart C., 2008. Genetic Variation of Stomatal Traits and Carbon Isotope Discrimination in Two Hybrid Poplar Families (*Populus deltoides* 'S9-2' × *P. nigra* 'Ghoy' and *P. deltoides* 'S9-2' × *P. trichocarpa* 'V24'). *Ann. Bot.*, **102,** 399-407.

Teixeira G., Monteiro A., Santos, C., Lopes, C.M., 2018. Leaf morphoanatomy traits in white grapevine cultivars with distinct geographical origin. *Ciência Téc. Vitiv.*, **33**, 90–101.

Tonietto J., Carbonneau A., 2004. A multicriteria climatic classification system for grape-growing regions worldwide. *Agric. For. Meteorol.*, **124**, 81-97.