Vitis **60**, 63–67 (2021) DOI: 10.5073/vitis.2021.60.63-67

Leaves of more cold hardy grapes have a higher density of small, sunken stomata

A. Nassuth[1)], M. A. Rahman[1), 2)], T. Nguyen[1)], A. Ebadi[1), 3)] and C. Lee[1)]

[1)] Department of Molecular and Cellular Biology, University of Guelph, Guelph/Ontario, Canada
[2)] Center for Viticulture and Small Fruit Research, Florida A&M University, Tallahassee/Florida, USA
[3)] Department of Horticulture, Faculty of Agriculture, University of Tehran, Karaj, Iran

Summary

Leaf stomatal density, index and size are known to be affected by the growing conditions, presumably to provide a better function for plant development. The question was whether there is a difference in stomatal parameters between grape species with different cold hardiness: *V. riparia* and *V. vinifera*; and the *V. vinifera* cultivars 'Riesling', 'Chardonnay', 'Sauvignon Blanc' and 'Merlot'. Analysis by scanning electron microscopy allowed the observation of 3 types of stomata in developing and mature leaves of all examined grape leaves. Stomatal parameters were found to be significantly affected by species or cultivar and growing conditions but not rootstock. A higher stomatal density and index were determined for the more cold hardy *V. riparia* and *V. vinifera* 'Riesling', whereby the higher number of stomata in 'Riesling' was found to be due to a higher number of small, sunken stomata. These findings might indicate a strategy of grape plants to optimize growth under low temperatures by using fast-acting stomata whose gas and water exchange are less affected than for larger stomata.

K e y w o r d s : *V. riparia*; *V. vinifera*; stomatal density; stomatal index; stomatal size; scanning electron microscopy.

Introduction

Stomata regulate the exchange of CO_2 and water vapour between plant leaves and the surrounding atmosphere (Hetherington and Woodward 2003). To balance the need for photosynthesis with the need for moderate water loss, plants adjust their stomatal aperture in response to short-term changes in water availability, temperature and atmospheric CO_2 concentration (Bertolino *et al.* 2019). Longer exposure to a stressful environment can lead to more permanent changes in newly developing leaves to reduce water loss. This includes changes in stomatal density (number of stomatal cells per unit area), stomatal index (ratio of the number of stomata to epidermal cells plus stomata x 100) or stomatal size (Xu and Zhou 2008, Bertolino *et al.* 2019). There is also a strong genetic component in the regulation of stomatal density in some species (Zhang *et al.* 2012). Key genes in the differentiation of epidermal cells to guard cells in Arabidopsis encode the transcription factors SCREAM (SCRM) and SCRM2 (Kanaoka *et al.* 2008). The same proteins, now named ICE1 and ICE2, respectively, are involved in the acquisition of cold hardiness (Wisniewski *et al.* 2014). This prompted us to ask the question whether there is a correlation between stomatal development and the cold hardiness of a plant.

Reports on grape stomata are limited. Leaf stomatal densities were found to vary and be characteristic for different *V. vinifera* cultivars grown under the same circumstances (Swanepoel and Villers 1987, Rogiers *et al.* 2009, Monteiro *et al.* 2013, Boso *et al.* 2016). Stomatal density was also reported to be sensitive to stressful environments in that it decreased with increasing soil temperature and atmospheric CO_2 (Moutinho-Pereira *et al.* 2009, Rogiers *et al.* 2011) or other environmental conditions (Gökbayrak *et al.* 2008 and references therein). Interestingly, light microscopy on sections and scanning electron microscopy (SEM) observations on whole tissues revealed that, compared to the grape leaf epidermal surface, stomata could be found raised above, at the same level and sunken (Pratt 1974, Swanepoel and Villiers 1987).

The current study reports on an SEM examination of stomata on leaves of various *V. riparia* accessions and *V. vinifera* cultivars and discusses the possible correlation between the types of stomata and the cold hardiness of the grape under investigation.

Material and Methods

P l a n t m a t e r i a l s a n d g r o w t h c o n d i t i o n s : Leaves were taken for observation from grape plants grown under two different conditions. For initial experiments, *V. vinifera* 'Riesling' (RL) and 'Chardonnay' (CH), and *V. riparia* accessions Manitoba (MB) and Quebec (QC), were maintained on their own roots in a growth chamber as described in Rahman *et al.* 2014. For cold treatment, the temperature was reduced to 4 °C, but otherwise identical conditions were maintained until sampling of leaves from these plants.

Correspondence to: Dr. A. Nassuth, University of Guelph, Molecular and Cellular Biology Department, 50 Stone Road, Guelph N1G 2W1 ON, Canada. E-mail: anassuth@uoguelph.ca

© The author(s).

This is an Open Access article distributed under the terms of the Creative Commons Attribution Share-Alike License (https://creativecommons.org/licenses/by/4.0/).

Later, *V. vinifera* 'Chardonnay' (CH), 'Riesling' (RL), 'Merlot' (ME) and 'Sauvignon blanc' (SB), grafted on 3309, Riparia Gloire or SO4 as rootstock, were sampled from two different vineyards (Stratus in the Niagara-Lakeshore sub-appellation, and Chateau des Charmes in the St. David's Bench sub-appellation) in Niagara Peninsula, Ontario, Canada. Sun-exposed canes (1 per plant) with developing leaves were collected from three to four vineyard plants per cultivar/accession or cv/rootstock combination) as soon as they had at least 12 nodes (July), in the middle (August) and at the end (September) of the growing season. These cultivars were chosen because of their importance for the winemakers in the area and their documented differences in bud hardiness throughout the dormant period, including acclimation and de-acclimation (November to April), with 'Riesling' being the most cold-hardy and 'Merlot' the least, and 'Chardonnay' and 'Cabernet Sauvignon' somewhere in-between (www.ccovi.ca/vine-alert).

Canes from growth chamber or field plants (1 per plant; at least three canes per accession/cultivar) were harvested and immediately placed in cool boxes, transported and stored in a 4 °C cold room until analysis of their leaves within 1 or 1 to 3 d, respectively. To allow for easy comparison between leaves at a similar developmental stage from canes of different species/accessions/cultivars with different numbers of nodes, leaves were numbered in such a way that the leaf harvested closest to the tip was named leaf 1 (suppl. Fig. S1; Hopper *et al.* 2014).

Analysis of grape leaves: Approximately 0.5 cm x 0.5 cm sections were cut from leaf positions that were similar to those suggested by Gökbayrak *et al*. (2008; region B) to have stable stomata counts under different conditions. Three to four images (together forming one biological replicate) were taken of the abaxial (lower) surface of each of these sections using a Hitachi TM-1000 Tabletop SEM at 300x or 600x magnification and projected on a computer screen (suppl. Fig. S1). LSM Image Browser was used to mark the stomata and pavement cells in one 0.0768 mm^2 area per image, and then ImageJ was used to count their number and perform size measures. The obtained data were used to calculate the stomatal density (number of stomata/ leaf area) and stomatal index (percentage, *i.e.* number of stomata/number of all cells in the same area x 100). Note that the youngest, first and second leaves of *V. vinifera* cultivars could not be analyzed due to the presence of too many long prostrate hairs on the epidermis (suppl. Fig. S2). In some cases, the length of all stomata and the maximal length of 30 pavements cells in an image were analyzed. The length of a stoma was defined as the length between the junctions of the guard cells at each end of the stoma (Xu and Zhou 2008). For initial analyses, leaves were digitally scanned along with a transparent ruler, and individual leaf areas were measured using ImageJ. The statistical significance of the differences between the means calculated for the various parameters was determined by one-way ANOVA and Tukey-Kramer HSD tests using the statistical software JMP 11 (SAS Institute). However, in the case of multi-factor ANOVA analysis and Tukey HSD post-hoc test, the statistical software package R was used (R Core Team 2019).

Results

Grape leaves contain different types of mature stomata: Leaf sizes were larger for the *V. riparia* accessions compared to those for corresponding leaves of the *V. vinifera* cultivars grown under identical conditions in growth chambers (Fig. 1; suppl. Tab. S1). For example, leaf 9 of *V. riparia* Manitoba and Quebec was at least 1.2 times larger than leaf 9 of *V. vinifera* 'Riesling' and 'Chardonnay'. Whereas most of the increase in leaf area took place after leaf 5, the total number of cells per unit area of the lower epidermis was already reduced to half by that time in most plants, except for 'Chardonnay', and did not differ much between leaf 9 and 10. This means that cell enlargement and not cell division predominated at later stages. Indeed, pavement cells were larger in older leaves compared to cells in younger leaves, with an average maximal length of 18.2 µm in leaf 10 vs 4.7 µm for leaf 3 of *V. riparia* Manitoba and 12.6 µm in leaf 10 *vs* 5.3 µm for leaf 3 of *V. vinifera* 'Riesling' (suppl. Tab. S2).

The adaxial surface of grape leaves does not contain any stomata or leaf hairs (suppl. Fig. S2). However, the abaxial surfaces of leaves from all the different nodes contain stomata of different sizes and levels relative to the epidermis: apparently smaller sunken stomata, medium stomata level with the leaf surface, and larger stomata higher up (Fig. 2, see also later). The maximal stomatal density (SD) and stomatal

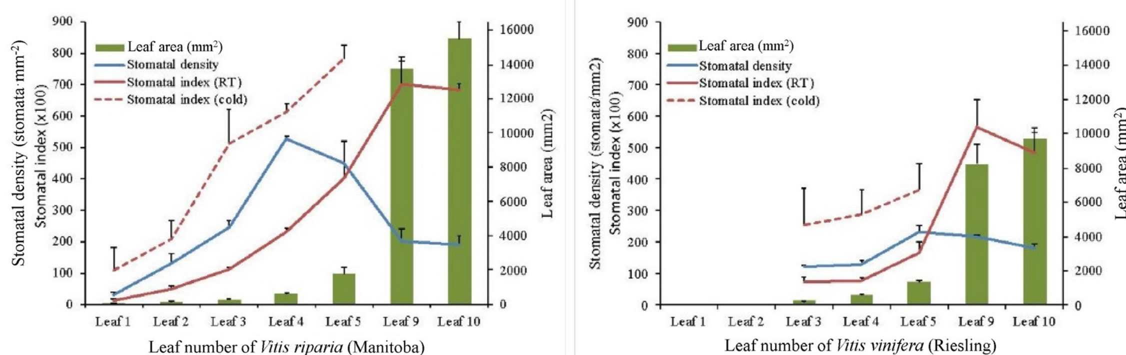

Fig. 1: Stomatal density (▬), stomatal index at room temperature (22 °C, ▬) and after cold treatment (4 °C, - - -) and leaf area (▬) for leaves taken from different nodes of *V. riparia* var. Manitoba (left) and *V. vinifera* 'Riesling' (right). Note that the leaf numbers are given to the leaves in ascending order from young to more mature leaves (see suppl. Fig. 1). Data shown are the means ± standard error of three biological replicates.

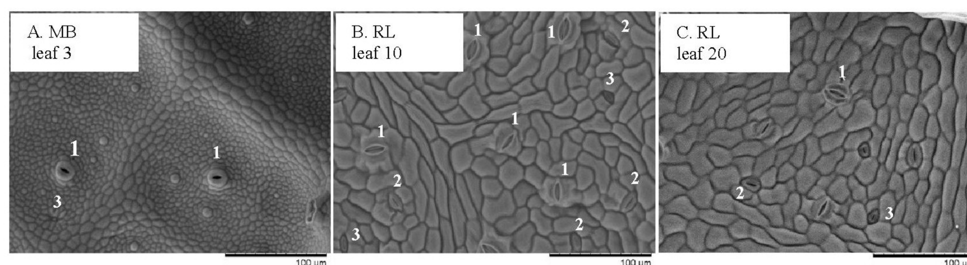

Fig. 2: Examples of SEM pictures showing the presence of large raised stomata (**1**), stomata level with the epidermal leaf surface (**2**), and small sunken stomata (**3**) on the abaxial surface of grape leaves taken from different nodes (3, 10 and 20). Note that the sunken stomata appear darker in the picture. MB = Manitoba, RL = 'Riesling'.

index (SI) were higher for the abaxial leaf surfaces of the *V. riparia* accessions than for those of the *V. vinifera* cultivars (Fig. 1, suppl. Tab. S1). Leaf SD increased up to leaf 3 or 4 in *V. riparia* species and leaf 4 or 5 in *V. vinifera* species and decreased steadily thereafter. SI, on the other hand, increased up to leaf 9 for both species and had decreased for leaf 10. These results suggest that epidermal cells of leaves older than leaf 9 or 10 no longer initiate divisions producing new stomata. Nevertheless, also these mature leaves contain stomata of different sizes (Fig. 2), showing that also mature stomata can be small.

Low temperature treatment increases the number of stomata: The stomatal density and index of young, developing leaves from growth chamber plants that were placed at 4 °C for six days increased substantially (suppl. Tab. S1). For example, leaf 3 from *V. riparia* Manitoba had an SD and SI of 527 mm^{-2} and 5.11 %, respectively when grown at 4 °C for 6 d, compared to 247 mm^{-2} and 1.11 % when grown under regular conditions (22 °C). Similarly, leaf 3 from *V. vinifera* 'Riesling' had an SD and SI of 191 mm^{-2} and 2.54 % at 4 °C compared to 124 mm^{-2} and 0.75 % at 22 °C. These increases were no longer observed for older leaves (leaves 4 and 5), presumably because most epidermal cells in these leaves had already committed to differentiating into a pavement cell when the cold treatment started. These results show that the commitment of dividing epidermal cells to become stomata increases upon low temperature treatment.

Stomatal parameters on mature leaves differ between vineyard-grown cultivars: Stomata were examined on 10th leaves collected at three different times during the season from grape cultivars grown on different rootstocks and at different sites, and the stomatal density and index were determined (Table). The multi-factor ANOVA analysis identified significant effects of cultivar, vineyard (on SI only) and time of season and their interactions on SD and SI (suppl. Tab. S3). The Tukey HSD test confirmed significant differences between the SD of cultivars, with the highest SD in 'Riesling' and the lowest in 'Merlot'. Also, the significant SI differences between cultivars were confirmed by the Tukey HSD test.

Analysis of stomatal sizes in July and September samples revealed that the length of all stomata varied from 8.3-47.3 µm, a range that is similar as was found for the growth chamber-grown plants (Fig. 3 and suppl. Fig. S3). However, the higher density of stomata in 'Riesling' com-

Table

Stomatal density and index of different grape cultivars grown on different rootstocks at Chateau des Charmes (CDC) and Stratus vineyards. Data shown are the mean of three biological replicates (SE = Standard error)

Vineyard	Cultivar	Root-stock	Stomatal density (stomata·mm^{-2}) (mean ± SE)			Stomatal index (×100) (mean ± SE)		
			July	August	September	July	August	September
CDC	Merlot	SO4	231.5 ± 21.8	248.8 ± 1.6	219.9 ± 18.5	7.1 ± 0.2	6.5 ± 0.3	6.2 ± 0.3
	Riesling	RipG	298.0 ± 20.0	328.4 ± 29.4	315.4 ± 29.0	8.4 ± 0.1	8.4 ± 0.3	7.9 ± 0.2
	Riesling	3309	327.0 ± 34.4	354.5 ± 46.5	300.9 ± 5.2	9.1 ± 0.7	8.3 ± 0.7	8.0 ± 0.9
	Riesling	SO4	277.8 ± 5.4	-	280.7 ± 22.6	7.8 ± 1.3	-	8.2 ± 0.5
	Chardonnay	SO4	295.1 ± 20.5	250.3 ± 22.6	282.1 ± 2.5	10.0 ± 0.7	8.2 ± 0.5	8.3 ± 0.6
	Sauvignon Blanc	SO4	222.8 ± 23.7	264.8 ± 22.6	231.5 ± 8.1	8.7 ± 0.5	8.5 ± 0.9	7.7 ± 0.6
Stratus	Merlot	SO4	178.0 ± 10.9	248.8 ± 25.4	178.0 ± 2.5	6.8 ± 0.5	9.6 ± 0.5	7.9 ± 0.3
	Merlot	RipG	202.5 ± 20.9	274.9 ± 26.1	222.9 ± 5.3	8.0 ± 0.1	9.1 ± 0.3	8.5 ± 0.3
	Merlot	3309	202.5 ± 22.9	259.0 ± 27.6	248.8 ± 20.9	7.5 ± 0.04	8.3 ± 0.3	7.5 ± 0.7
	Riesling	SO4	276.3 ± 12.0	332.8 ± 23.8	273.0 ± 22.6	9.4 ± 0.04	8.0 ± 0.1	8.2 ± 0.6
	Chardonnay	3309	243.1 ± 10.7	345.8 ± 35.6	225.7 ± 2.5	8.2 ± 0.1	9.9 ± 0.4	7.8 ± 0.3
	Sauvignon Blanc	RipG	225.7 ± 23.1	219.9 ± 14.3	224.2 ± 1.4	8.5 ± 0.7	8.6 ± 0.3	8.8 ± 0.2

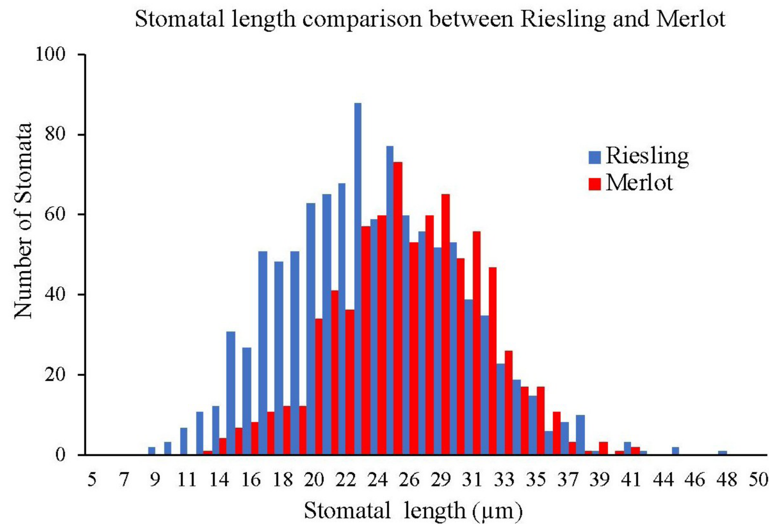

Fig. 3: Length of stomata present in the 10th leaves from vineyard-grown *V. vinifera* cultivars. Stomata were analyzed from September leaves of 'Riesling' (n = 1047) and 'Merlot' (n = 767).

pared to 'Merlot' was mainly due to additional small stomata (Fig. 3). Independent re-analysis of the sunken stomata only, on the September pictures, confirmed that all small stomata are sunken stomata (Fig. 4 and suppl. Fig. S4). The length of these sunken stomata varied between 8.9 and 28.2 µm and comprised 33.5, 22.7, 11.2 and 15.8 % of the total stomata present on leaves of resp. 'Riesling', Chardonnay', Sauvignon blanc' and 'Merlot' (Fig. 4 and suppl. Fig. S4).

Discussion

This study confirmed the presence of three types of stomata: small-sized sunken, medium-sized at the same level, and larger stomata which are raised above the other epidermal cells (SWANEPOEL and VILLERS 1987, MONTEIRO *et al.* 2013), in grape leaves of all ages. The stomatal densities which were determined for leaf 9 are in the same range as the 207-286 mm^{-2} reported for leaf 8 of *V. vinifera* cultivars by MONTEIRO *et al*. 2013. Total cell density was lower, and pavement cell size was larger in older leaves compared to younger leaves, suggesting that the cells in these leaves no longer divide but elongate instead. Older leaves also do not produce new stomata anymore, as reflected by their decreased stomatal index. Taken together these data mean that leaves are mature by leaf 8 to 9, in line with what had been reported by MONTEIRO *et al*. (2013) and HOPPER *et al*. (2014). For further studies on mature stomata we therefore chose leaf 10.

Leaves from the more cold hardy *V. riparia* appeared to contain more stomata than leaves from the less cold hardy *V. vinifera*, and cold treatment in growth chambers appeared to increase the number of stomata in both. This prompted a more extensive analysis of vineyard-grown cultivars. The results showed that leaves from 'Riesling', a more cold hardy cultivar, have a higher stomatal density than leaves from 'Merlot', a less cold hardy cultivar. This finding appears to be counterintuitive, as one would think that having more stomata gives the opportunity for more water loss, and as a result, is less favourable. However, the addition of small, sunken stomata can be advantageous. First, smaller stomata are thought to open and close faster compared to larger stomata, while using less energy, and thus be able to open under conditions where larger stomata stay closed (HETHERINGTON and WOODWARD 2003, DRAKE *et al*. 2013). Second, the smaller stomata are located below the leaf surface, decreasing the boundary layer conductance and thus water loss would be less affected by environmental conditions. Therefore, the observation by HOPPER *et al*. (2014) of a lower water loss by *V. riparia* (Riparia Gloire) leaves than by *V. vinifera* ('Shiraz') leaves under stress conditions might in part be because of differences in their stomata complement. Another reason that the additional small stomata might be advantageous is that they might compensate for the lower diffusion of

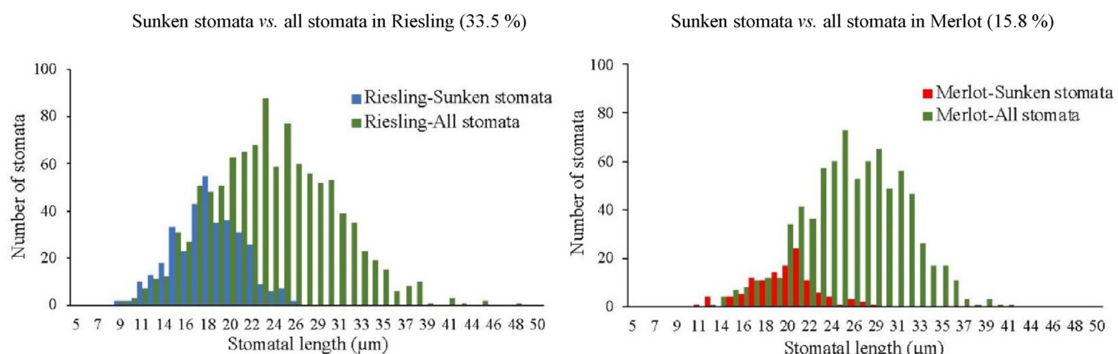

Fig. 4: Sunken stomata are small. The length of sunken stomata was determined by an independent re-analysis of the pictures used for Fig. 3 and the results were superimposed on the initial data for all stomata.

CO_2 at lower temperatures. A relation between stomatal size and stress tolerance is supported by observations from MONTEIRO *et al*. (2013). We calculated, based on their data, that the more drought sensitive grape cultivar, 'Aragonez', had fewer small stomata (about 32 %) compared to drought tolerant cultivars (about 60-64 %). According to this line of thinking, it is not surprising that lower temperatures can induce the presence of more small stomata, as was observed for developing leaves from grape plants that were placed under low temperature conditions (Fig. 1).

More data are needed to resolve the effect of rootstock and site on stomatal development. Nevertheless, the data shown here suggest that a possible effect should be considered in any future comparative studies on stomata.

Conclusion

The data reported here show that grape leaves contain different types of stomata and that additional small, sunken stomata are present in more cold hardy grape plants. The data therefore give fuel to the speculation that having such stomata is advantageous for plants under low temperature conditions, and regulation of their development could be a strategy grape plants use to maximize growth under more stressful conditions. The very small, sunken stomata are not captured when making imprints with nail polish or similar materials, a commonly used method to analyze leaf stomata in a variety of plants (HAMANISHI *et al.* 2012, MONTEIRO *et al*. 2013, BOSO *et al*. 2016, NEUFFER *et al*. 2018). Thus, it remains unclear how widespread small, sunken stomata are in other plant species. It also remains uncertain if stomatal development is affected when plants are exposed to drought or cold conditions. Further SEM analyses of leaves from various plants would be needed to clarify the situation.

Acknowledgements

We thank Stratus vineyards and Chateau des Charmes for allowing us to collect canes from their vineyards. Dr. H. FISHER supplied access to the *V. riparia* accessions. Financial assistance was provided by grants from NSERC and the Ontario Grape and Wine Industry.

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. DOI: https://doi.org/10.3389/fpls.2019.00225

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. DOI: http://dx.doi.org/10.5073/vitis.2016.55.17-22

DRAKE, P. L.; FROEND, R. H.; FRANKS, P. J.; 2013: Smaller, faster stomata: scaling of stomatal size, rate of response, and stomatal conductance. J. Exp. Bot. **64**, 495-505. DOI: https://doi.org/10.1093/jxb/ers347

GÖKBAYRAK, Z.; DARDENIZ, A.; BAL, M.; 2008: Stomatal density adaptation of grapevine to windy conditions. Trakia J. Sci. **6**, 18-22 (https://www.semanticscholar.org/paper/STOMATAL-DENSITY-ADAPTATION-OF-GRAPEVINE-TO-WINDY-G%C3%B6kbayrak-Dardeniz/d8be0074db8645ac30b1489296f26668dc92a8da).

HAMANISHI, E. T.; THOMAS, B. R.; CAMPBELL, M. M.; 2012: Drought induces alterations in the stomatal development program in *Populus*. J. Exp. Bot. **63**, 4959-4971. DOI: https://doi.org/10.1093/jxb/err313

HETHERINGTON, A.; WOODWARD, F. I.; 2003: The role of stomata in sensing and driving environmental change. Nature **424**, 901-907. DOI: https://doi.org/10.1038/nature01843

HOPPER, D. W.; GHAN, R.; CRAMER, G. R.; 2014: A rapid dehydration leaf assay reveals stomatal response differences in grapevine genotypes Hortic. Res. **1**, 2. DOI: https://doi.org/10.1038/hortres.2014.2

KANAOKA, M. M.; PILLITTERI, L. J.; FUJII, H.; YOSHIDA, Y.; BOGENSCHUTZ, N. L.; TAKABAYASHI, J.; ZHU, J. K.; TORII, K. U.; 2008: SCREAM/ICE1 andSCREAM2 specify three cell-state transitional steps leading to Arabidopsis stomatal differentiation. Plant Cell **20**, 1775-1785. DOI: https://doi.org/10.1105/tpc.108.060848

MONTEIRO, A.; TEIXEIRA, G.; LOPES, C. M.; 2013: Comparative leaf micromorphoanatomy of *Vitis vinifera* ssp. *vinifera* (Vitaceae) red cultivars. Cienc. Tec. Vitivinic. **28**, 19-28 (http://www.scielo.mec.pt/scielo.php?script=sci_abstract&pid=S0254-02232013000100003&lng=en&nrm=iso).

MOUTINHO-PEREIRA, J. M.; GONÇALVES, B.; BACELAR, E.; CUNHA, J. B.; COUTINHO, J.; CORREIA, C. M.; 2009: Effects of elevated CO_2 on grapevine (*Vitis vinifera* L.): physiological and yield attributes. Vitis **48**, 159-165. DOI: https://doi.org/10.5073/vitis.2009.48.159-165

NEUFFER, B.; WESSE, C.; VOSS, I.; SCHEIBE, R.; 2018: The role of ecotypic variation in driving worldwide colonization by a cosmopolitan plant. AoB Plants **10**, 1. DOI: https://doi.org/10.1093/aobpla/ply005

PRATT, C.; 1974: Vegetative anatomy of cultivated grapes – a review. Am. J. Enol. Vitic. **25**, 131-150 (https://www.ajevonline.org/content/25/3/131).

RAHMAN, M. A.; MOODY, M. A.; NASSUTH, A.; 2014: Grape contains 4 *ICE* genes whose expression includes alternative polyadenylation, leading to transcripts encoding at least 7 different ICE proteins. Environ. Exp. Bot. **106**, 70-78. https://doi.org/10.1016/j.envexpbot.2014.01.003

R CORE TEAM; 2019: R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria (https://www.R-project.org/).

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. DOI: https://doi.org/10.1093/jxb/erp217

ROGIERS, S. Y.; HARDIE, W. J.; 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. DOI: https://doi.org/10.1111/j.1755-0238.2011.00124.x

SWANEPOEL, J. J.; VILLIERS, C. E.; 1987: A numerical-taxonomic classification of *Vitis* spp. and cultivars based on leaf characteristics. S. Afr. J. Enol. Vitic. **8**, 31-35. DOI: https://doi.org/10.21548/8-2-2311

WISNIEWSKI, M; NASSUTH, A; TEULIERES, C.; MARQUE, C.; ROWLAND, J.; CAO, P. B.; BROWN, A.; 2014: Genomics of cold hardiness in woody plants. Crit. Rev. Plant Sci. **33**, 92-124. DOI: http://dx.doi.org/10.1080/07352689.2014.870408

XU, Z; ZHOU, G.; 2008: Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. J. Exp. Bot. **59**, 3317-3325. DOI: https://doi.org/10.1093/jxb/ern185

ZHANG, L.; NIU, H.; WANG, S.; ZHU, X.; LUO, C.; LI, Y.; ZHAO, X.; 2012: Gene or environment? Species-specific control of stomatal density and length. Ecol. Evol. **2**, 1065-1070. DOI: https://doi.org/10.1002/ece3.233

Received October 1, 2020
Accepted January 19, 2021