LEAF MORPHOANATOMY OF PORTUGUESE AUTOCTONES WHITE GRAPEVINE CULTIVARS OF DIFFERENT GEOGRAPHICAL ORIGIN

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Abstract

The knowledge of grapevine varieties leaf morphoanatomy is an important tool to understand the *taxa* ability to adapt and produce under biotic and abiotic stresses. Aiming to characterize and discriminate between four white Portuguese grapevine (*Vitis vinifera* subsp. *vinifera*) varieties – 'Alvarinho' (Al), 'Arinto' (Ar), 'Encruzado' (En) and 'Viosinho' (Vi) - from different Winegrowing Regions (Vinhos Verdes, Lisboa, Dão and Douro, respectively), grown side by side in field conditions -, leaf morphoanatomic characteristics were studied under light (LM) and scanning electron microscopy (SEM). The individual primary leaf area revealed significant differences between all cultivars, with the highest value presented by 'Arinto' and the lowest by 'Viosinho', while 'Encruzado' and "Alvarinho" gave intermediate values. Nevertheless, no significant differences were detected on leaf specific dry weight which can be explained by the quite different mesophyll structure. 'Arinto' presented the lowest values for total thickness of the lamina, thickness of palisade and spongy parenchyma. The length and thickness of upper and lower epidermal cells of the four cultivars were similar. Under SEM magnification three types of stomata were identified in all the studied genotypes: sunken, at the same level, and raised above the other epidermal cells. No significant differences were registered between cultivars for same-level and sunken stomata values and for stomatal density. 'Alvarinho' showed the highest percentage of total for raised-above stomata and 'Viosinho' the lowest values. In conclusion, the data indicate some differences in leaf morphoanatomy between grapevine cultivars. Are these grapevine leaf traits differences – e.g. stomata type and mesophyll structure – involved in the differential behavior observed under field conditions? Further studies are needed.

Key words: grapevine, leaf, epidermis, stomata, mesophyll

1 INTRODUCTION

Grapevine cultivars have been reported to adapt to water deficit, mainly by modifying their morphological and anatomical characteristics (Gómez-del-Campo et al. 2003; 2004; Koundouras et al. 2008; Costa et al. 2012). Grapevine cultivar susceptibility and resistance to downy mildew associated with differences in leaf morphology at the macro- and microscopic levels were studied by Boso et al. (2010; 2011). Few attempts have been made to employ leaf micromorphology traits to explain heat and water stress tolerance (Gómez-del-Campo et al. 2003; Koundouras et al. 2008; Costa et al. 2012). Recently, Sadras et al. (2012) reported that stomata density of the 'Shiraz' cultivar was unaffected by temperature, but stomata length and width increased with heat. They concluded that longer and wider stomata contributed to the enhanced plasticity of stomatal conductance under higher temperatures.

Monteiro et al. (2013) concluded that the most important micromorphological characteristics for distinguishing between grapevine genotypes were stomatal frequency and indument distribution.

The present study aims to see whether the leaf morphoanatomic characteristics of *V. vinifera* subsp. *vinifera* white varieties "Alvarinho", "Viosinho", "Encruzado" and "Arinto", analyzed under LM and SEM can be used do distinguish between the different genotypes.

2 MATERIALS AND METHODS

Eight-year-old field-grown grapevines of the white Portuguese grapevine (*Vitis vinifera* subsp. *vinifera*) cultivars – 'Alvarinho', 'Arinto', 'Encruzado' and 'Viosinho' - from different Portuguese Winegrowing Regions (Vinhos Verdes, Lisboa, Dão and Douro, respectively). These are kept in field conditions in an experimental area at Tapada da Ajuda, Lisbon.

During 2013 full-expanded leaves from each variety were collected from the 8th node of the shoot, at veraison (end of July). Individual primary leaf area was estimated using the methodologies proposed by Lopes and Pinto

(2000). Leaf dry weight and specific dry weight were assessed at veraison in a random sample of 12 leaves (8th node), with the leaf area estimated by the length of leaf veins.
Some of the collected leaves were selected for the morphoanatomical studies. Several small leaf blade sections were cut from the central leaf part, between the L1 and L2 veins. All measurements and counts related with morphoanatomical traits were done on random fields under light (LM) and scanning electron microscopy (SEM), always at comparable leaf situations and magnifications.
For morphoanatomical analysis small pieces of fresh leaves of each cultivar were fixed in a 5% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.0, for 4h at 4°C, according to the usual procedures (Hayat, 1981). Samples were washed and dehydrated in an ascendant ethanol series and processed using the paraffin micro-technique (Ruzin, 1999). Transverse sections were sectioned at 8-10 μm, on a Leitz 1512 Minot microtome and examined with a Nikon Labophot 2 photomicroscope. Images were obtained with a Nikon FX-35W camera equipped with a semi-automatic Nikon PFX adapter (Nikon®). The anatomical observations focused on the total lamina thickness, thickness of palisade and spongy parenchyma, thickness of upper and lower epidermal cells and thickness of upper cuticle.
For scanning electron microscopy (SEM), plant material was fixed as above, critical-point dried on a Critical Point Polaron BioRad E3500, and coated with gold in a Jeol JFC-1200. Observations were carried out at 15 kV on a Jeol JSM-5220 LV scanning electron microscope equipped with a direct image acquisition system. Measurements and counts were obtained by computer-assisted image analysis. SEM observations focused on the upper and lower epidermis surface details – type of indumentum, epicuticular waxes, stomata density (stoma mm^{-2}), and type of stomata and percentage of total of each type, and stomata cell length and width. The type of stomata followed the nomenclature given by Pratt (1974), who considered 3 types: raised above, at the same level, and sunken to the level of the other epidermal cells.
Anatomical data were analyzed by one-way ANOVA in accordance with GLM procedures from the SAS® program package (SAS Institute, Cary, NC, USA), and statistical differences between means were assessed by LSD test ($P < 0.05$).

3 RESULTS AND DISCUSSION

Tables 1 and 2 summarise the leaf morphoanatomic characteristics of the four *V. vinifera* subsp. *vinifera* white Portuguese varieties. Individual primary leaf area reveals significant differences between the four genotypes, with the highest value showed by 'Arinto' and the lowest by 'Viosinho'. The highest dry weight value was also showed by 'Arinto', whilst 'Alvarinho' did not present significant differences for the other two varieties – Vi and 'Encruzado' (Table 1).
Transverse sections showed uniform, regular and slightly polygonal epidermal upper cells, with no significant difference between varieties. They had almost the same rectangular or slightly polygonal cell shape – features similar to those reported by Gallet (2000), Boso et al. (2010) and Monteiro et al. (2013). The studied varieties significantly differ in the total thickness of lamina and thickness of spongy tissue (Table 1). Arinto' presented the lowest values for total thickness of the lamina, thickness of palisade and spongy parenchyma, significantly different from the other three varieties. The same differences were also observed between other cultivars by Monteiro et al. (2013).
Under SEM magnification all the genotypes exhibited three types of stomata (Figure 1): sunken, at the same level, and raised above the other epidermal cells that were also reported by Swanepoel and Villers (1987) and Monteiro et al. (2013). No significant differences were registered between cultivars for same-level and sunken stomata values. Nevertheless, 'Alvarinho' showed the highest percentage of total for raised-above stomata and 'Viosinho' the lowest values. Stomatal density (nº mm^{-2}) does not differ significantly between the four white grapevine varieties, with values of the same order of magnitude as those presented for several other *Vitis* genotypes by Swanepoel and Villers (1987) and Monteiro et al. (2013). Raised and at same level stomata only showed differences in the stomata width, whereas the sunken ones showed differences in the length (Table 2). Boso et al. (2011) also referred significant differences between the genotypes in terms of stomatal length and width, but they do not distinguish the three types of stomata.

4 CONCLUSIONS

LM and SEM leaf images revealed that micromorphoanatomical features of the grapevine Portuguese white varieties 'Alvarinho', 'Arinto, 'Encruzado' and 'Viosinho' differ significantly. The leaf traits that contributed most to distinguishing between grapevine cultivars were: leaf area, leaf dry weight, mesophyll thickness (total mesophyll, palisade and spongy tissues), type of stomata (%) and size of stomata cells.

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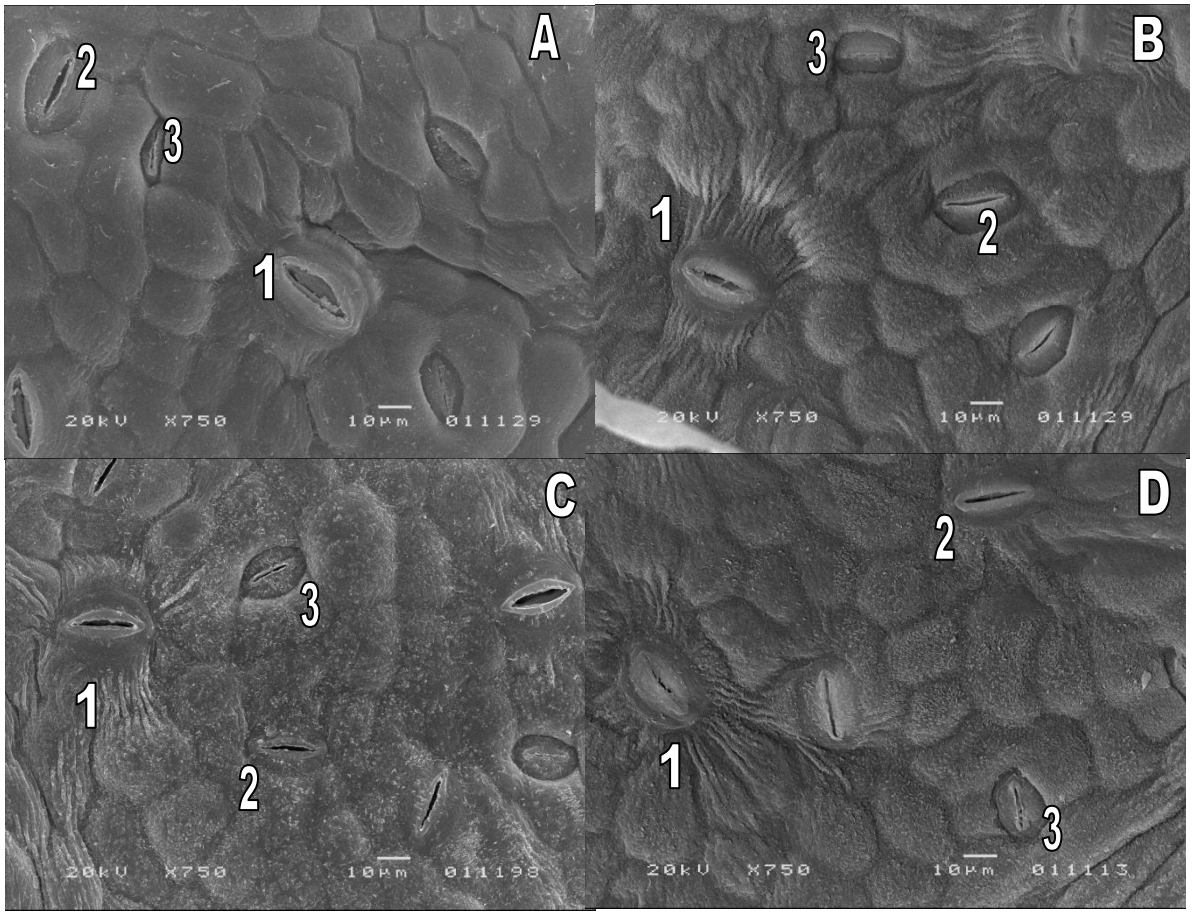

Figure 1 - Scanning electron micrographs of the lower epidermis surface of mature leaves of *V. vinifera* subsp. *vinifera*. A - 'Encruzado'; B - 'Alvarinho'; C – 'Viosinho'; D- 'Arinto' - There are also different types of stomata: (1) raised above; (2) at the same level; (3) sunken (x750).

Table 1 - Means and standard error (in parentheses) of several leaf morphoanatomical features of four Portuguese white *V. vinifera* subsp. *vinifera* varieties, at veraison.

Leaf character	'Alvarinho'	'Arinto'	'Encruzado'	'Viozinho	sig.
Macromorphological parameters					
Individual primary leaf area (cm^2)	240.1 (13.42) c	378.9 (23.15) a	309.3 (16.69) b	224.0 (16.45) c	***
Dry weight (g)	1.3 (0.11) bc	2.1 (0.15) a	1.6 (0.09) b	1.2 (0.14) c	***
Specific dry weight (DW/LA; mg cm^{-2})	5.2 (0.23)	5.6 (0.32)	5.1 (0.30)	5.0 (0.29)	ns
Micromorphoanatomical characters					
Total thickness of lamina (µm)	208.26 (5.34) a	184 (4.17) b	214.25 (6.67) a	221.25 (5.37) a	***
Total thickness of parenchyma	174.2 (5.09) a	144.25 (4.27) b	182.5 (5.97) a	184.25 (4.78) a	***
Thickness of palisade tissue (µm)	65.26 (2.55) a	58.25 (3.2) b	74.25 (3.63) a	61.50 (2.68) b	**
Thickness of spongy tissue (µm)	111.54 (2.88) a	85.25 (2.64) b	106.25 (4.73) a	122.75 (2.64) a	***
Thickness of upper cuticle (µm)	2.36 (0.10)	1.93 (0.14)	2.35 (0.07)	2.23 (0.10)	ns
Thickness of lower cuticle (µm)	2.55 (0.04)	2.47 (0.08)	2.4 (0.06)	2.37 (0.11)	ns
Thickness of upper epidermal cells (µm)	16.38 (0.81) ab	15.5 (1.02)	17.50 (1.66)	20.25 (1.12)	ns
Thickness of lower epidermal cells (µm)	19.76 (0.82)	20.75 (1.08)	19.5 (1.74)	18.75 (1.00)	ns

[a] Means of 12 replicates.

In each row different letter suffixes show statistically significant differences at P<0.05 by LSD test. sig – significance; ns – not significant; ** - significant at P<0.01and *** - significant at P<0.001.

Table 2 - Means and standard error (in parentheses) of stomatal density, raised aboe, at the same level ans sunken stomata types, stomata length and width of four Portuguese white *V. vinifera* subsp. *vinifera* varieties, at veraison.

Leaf character		'Alvarinho'	'Arinto'	'Encruzado'	'Viozinho	sig.
[a]Stomatal density (nº mm^{-2})		172.9 (16.25)	170.4 (12.59)	217.9 (22.21)	200.6 (11.93)	ns
[a]Stomata type (% of total)	Raised above	42.8 (0.10) a	33.0 (0.05) ab	26.0 (0.05) ab	21.5 (0.05) b	*
	Same Level	47.0 (0.11)	45.9 (0.08)	54.7 (0.09)	51.4 (0.06)	ns
	Sunken	10.2 (0.06)	21.1 (0.06)	19.4 (0.05)	27.1 (0.07)	ns
Raised above (µm)	Length	31.9 (0.64)	30.50 (1.23)	33.4 (0.85)	30.4 (1.86)	ns
	Width	23.1 (0.45) ab	21.40 (0.86) bc	24.5 (1.04) a	19.3 (1.65) c	**
Same Level (µm)	Length	26.6 (0.80)	26.3 (0.93)	27.5 (0.85)	26.3 (0.65)	ns
	Width	17.5 (0.91) a	14.9 (0.50) b	17.9 (0.82) a	16.4 (0.70) ab	**
Sunken (µm)	Length	24.4 (1.09) a	21.9 (1.30) ab	18.7 (1.18) c	20.2 (0.68) bc	***
	Width	11.4 (0.89)	12.2 (0.76)	9.8 (0.73)	11.6 (0.89)	ns

[a] Means of 12 scanning electron micrographs: x 750;

In each row different letter suffixes show statistically significant differences at P<0.05 by LSD test. sig – significance; ns – not significant; *- significant at P<0.05 ** - significant at P<0.01and *** - significant at P<0.001.