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Research Note

Variability of the stomata among 'Albariño' (*Vitis vinifera* L.) clones and its relationship with susceptibility to downy mildew

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Introduction: In grapevines the stomata are located on the abaxial epidermis of the leaves, arranged in no specific order. As in other plants, they regulate the communication between the internal tissues and the external atmosphere, playing a critical role in both photosynthesis and transpiration processes. Moreover, stomata are the main entrance for pathogens such as *Plasmopara viticola* (Berk & Curt.) Berl. & de Toni, the causal agent of downy mildew, one of the most widespread fungal diseases of grapevines. Due to this and the fact that downy mildew causes large losses in yield and quality of grapes, there is a great interest to determine the causes of the different degree of susceptibility to this pathogen. Some authors have searched for anatomical features that might be related to the penetration and development of *P. viticola* in the mesophyll of grapevine leaves, such as the density of leaf hairs (KORTEKAMP and ZYPRIAN, 1999) or the ultrastructure of stomata (JÜRGES *et al.* 2009). Other authors have examined the differences concerning the number of stomata in different grapevine cultivars (DÜRING 1980, PALLIOTTI *et al.* 2000, BEN SALEM-FNAYOU *et al.* 2005), but none had evaluated the differences for this aspect between clones of the same cultivar. Recently some authors have found a strong correlation between the number and size of stomata and the susceptibility to downy mildew (LU *et al.* 2010). The aim of this work is to determine whether different clones of 'Albariño' differ in terms of their number and size of stomata and its possible relation with the different susceptibility to *P. viticola* of these same clones.

Material and Methods: Eight clones of 'Albariño' (*Vitis vinifera* L.) from the collection of the *Misión Biológica de Galicia* (CSIC), known as CSIC-1, CSIC-2, CSIC-3, CSIC-6, CSIC-7, CSIC-9, CSIC-10 and CSIC-11, were used in this study. When most of their green shoots presented between 12 and 14 internodes (around bloom, June), the 8th leaf from the base of a fertile green shoot was collected from five plants per clone during three consecu-

tive years (2005, 2006 and 2007). A print (approx. size 1 x 1 cm) obtained with transparent nail polish from the underside of each leaf was then made, close to the petiole sinus and between the main vein and the left lateral vein, according to D'AMBROGIO DE ARGÜESO (1986) method. Each print was observed using a light microscope (Nikon Eclipse E200) at 40 x magnification and the number of stomata per 0.196 mm² was recorded in triplicate for all sampled leaves. Moreover, the size (width and length) of 50 stomata per clone and year was measured on these prints, using the NIS-Elements Basic Research v 2.34 Software (Nikon Instruments Inc., Melville, U.S.A.). Also during 3 years, the susceptibility of these same clones to *P. viticola* was evaluated in the field. Therefore, the severity disease index was recorded as the sum of the surface area of all disease spots/total leaf surface area, according to Boso *et al.* (2004).

The differences between the studied variables were analysed by ANOVA and Fisher's protected least significant difference (LSD) test. All analyses were performed using the SAS System v 9.1 software package.

Results and Discussion: The variable "number of stomata" was significantly ($P < 0.01$) influenced by the interaction clone \times year, therefore the mean number of stomata depends on the meteorological conditions of the year. Effectively, in the year 2007, the mean number of stomata was significantly higher (35 stomata/0.196 mm²) than in 2006 (33 stomata/0.196 mm²) or in 2005 (32 stomata/0.196 mm²). This might be related to the lower temperatures of June and July (the months of maximum leaf development) of 2007 (18.1 °C, compared to 21.0 °C in 2006 and 20.3 °C in 2005), as well as to the highest precipitation recorded in these months (261 mm for 2007, compared to 16.2 mm and 60.9 mm respectively in 2006 and 2005) according to other authors who stated less leaf development under water stress conditions, low irradiance regimes or extremely high temperatures (PALLIOTTI *et al.* 2000; GÓMEZ DEL CAMPO *et al.* 2003). The analysis of the number of stomata per year revealed significant differences within the clones for the years of study 2005 and 2007, but no significant differences for 2006 (Table). This shows the number of stomata differs among cultivars, as well as within clones of the same cultivar. Regardless of the year interaction, the clones CSIC-6 and CSIC-9 always presented the largest number of stomata, while CSIC-3 always presented the fewest.

Concerning stomata size, there were no significant differences for their length. They were only significant for their width and moreover, they were significant among the years of study (Table). However, regardless of the year, the clones CSIC-7 and CSIC-3 presented always the widest stomata.

For the susceptibility to downy mildew, CSIC-11 and CSIC-9 were the most susceptible clones to this pathogen with 0.06 and 0.05, respectively, of disease severity (Table). On the other hand CSIC-3 was the least susceptible with 0.02 for this parameter.

Regardless of the year, the clone CSIC-9 always presented the largest number of stomata, being also the most susceptible to *P. viticola*. Considering that stomata repre-

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Table

Mean values and least significant differences (LSD) for the stomata number and width and the severity disease index of the different clones

Clones	N° stomata/0.196 mm ²			Stomata width (µm)			Disease severity** 3 years
	2005	2006	2007	2005	2006	2007	
CSIC-1	29 CD*	34 AB	36 ABCD	18.17 C	20.01 ABC	19.97 ABC	0.036 CD
CSIC-2	33 AB	32 AB	34 BCD	19.75 BC	18.46 C	18.89 BC	0.043 BC
CSIC-3	27 D	34 AB	33 CD	20.96 AB	20.32 A	20.28 AB	0.022 E
CSIC-6	36 A	32 AB	38 A	19.57 BC	20.33 A	18.68 C	0.028 DE
CSIC-7	30 BCD	31 B	34 BCD	21.59 A	21.20 A	20.65 A	0.042 BC
CSIC-9	33 AB	35 A	37 AB	19.72 BC	18.67 BC	20.49 A	0.050 AB
CSIC-10	33 AB	35 A	32 D	19.79 B	20.29 AB	19.64 ABC	0.035 CD
CSIC-11	32 BC	33 AB	36 ABC	20.20 AB	21.43 A	18.71 C	0.058 A
LSD (0.05)	3.66	3.50	3.70	1.60	1.63	1.45	0.011

* Means with the same letter are not significantly different

** Sum of the surface area of all disease spots/leaf surface area

sent the main entrance for the pathogen (ALLÈGRE *et al.* 2006), the results of this work hint a relationship between stomata number and downy mildew susceptibility rather than with stomata size. Although this correlation was not always given and this is not the only factor that influences in the susceptibility to this pathogen, an apparent effect of the stomata frequency seems to exist. Thus, further studies should be developed in this way in order to clarify this aspect.

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