STOMATAL BEHAVIOUR OF THREE MINORITY GRAPEVINE VARIETIES GROWN IN THE LA MANCHA REGION (SPAIN)

LE COMPORTÉMENT STOMATIQUE DE TROIS VARIÉTÉS DE VIGNE CULTIVÉES DANS LA RÉGION DE LA MANCHA (ESPAGNE)

J.L. Chacón and J. Martínez
Instituto de la Vid y del Vino de Castilla-La Mancha. Ctra. de Albacete, s/n.13.700-Tomelloso (Spain)
jlchacon@jccm.es

ABSTRACT
Stomatal conductance (g_s), net photosynthesis (A_N) and leaf water potential (Ψ_{leaf}) in 3 grapevine cultivars were measured from June to September in 2008 and 2009. Significant differences in Ψ_{leaf} and intrinsic water use efficiency (WUE_i) were found between varieties for the same ranges of g_s. Moravia Agria and Coloraillo showed high stomatal sensitivity to water stress. In contrast, Tinto Velasco behaved like a less sensitive variety; Ψ_{leaf} fell up to -2.1 MPa. Tinto Velasco displayed the highest water use efficiency levels. However, Coloraillo kept stomata open, even in hours of the day when the water deficit was more acute, and was therefore a less efficient cultivar in terms of water use.

KEYWORD
gas exchange – intrinsic water use efficiency – leaf water potential – stomatal conductance

INTRODUCTION
Stomatal closure is the dominant factor limiting gas exchange during water deficit. Stomata regulate transpiration so that sufficient carbon is gained while leaf water potential (Ψ_{leaf}) is prevented from becoming too negative and the break-down of the plants hydraulic system is avoided (Schultz, 2003).

Nowadays is thought that differences in stomatal control of varieties are due to differences in the perception of abscisic acid [ABA]. This chemical signal come from the roots and it is transported up to the leaves across the xilema by means of a mechanism not elucidated totally (McDonald, Davies, 1996). The synthesis of this hormone in the roots increases with the decrease of the soil water status.

Differences in the sensitivity of stomata to environmental factors, such as vapour pressure deficit (VPD) and/or water deficit, seem to be modulated by [ABA], but seem that hydraulic signalling may also play a role. Both factors may actually partly depend on each other in the regulation and recovery of stomatal conductance (g_s) during and after a water stress. Nitric oxide (NO) may be another signalling molecule involved in stomatal responses to drought (Schultz, Stoll, 2010). Stomatal sensitivity to [ABA] can change depending on the environmental conditions and on the plant status; also it seems to depend on adjustment factors (Medrano, Flexas, 2004). Different stomatal behavior between grapevine cultivars have been demonstrated by many authors (Schultz, 1996; Bota *et al*., 2001, Gómez del Campo *et al*., 2004, Ojeda *et al*., 2005).

A few years ago, the vine heritage in La Mancha region was made up by tens of varieties. As a result of the restructuring plans, over the last years it has been seen a loss of autochthonous grapevine varieties traditionally grown in this region. Some of them are still unknown from the physiological point of view and they would be able to be used to improve varieties by means of genetic engineering.

With the aim to learning more about some of these cultivars, this paper presents the results of the stomatal behavior of three minority grapevine varieties, and in particular its effects on water use efficiency.

MATERIALS AND METHODS

Environmental conditions and plant material

The study was performed in 2008 and 2009 on a multivarietal vineyard over the summer season. The plantation of the plot with the rootstock 110-R dated from 2002. The vines, grown on trellises, at a distance of 3 m x 1.5 m (row by vine spacing), were trained to a double cordon Royat system, with 3 spurs of 2 buds on each arm and 120ºE-SE/300ºW-NW oriented. The vineyard was maintained by a drip irrigation system with two drips per plant.

The plot was situated on the plain that forms the alluvial fan of the River Guadiana. According to soil taxonomy, the soils are Calcixerollic-Petrocalcic Xerochrepts with limestone encrusting below 35-40 cm in depth (Montero, Brasa, 1998).

In 2008 and 2009 total rainfall over the season was 52.1 l/m^2 and 30 l/m^2, respectively; the mean maximum temperatures were 30.2ºC and 32.8ºC, respectively, and mean minimum temperatures were 14.7ºC and 16.3ºC, respectively.

Three red minority varieties were studied: Coloraillo, Moravia Agria and Tinto Velasco. The study was carried out on all the data obtained (between 134 and 141 measurements per variety) and the two years, the water conditions of the plot during ripening were moderate-strong water constraint (Ψ_{PD} remained over the season between -0.25 MPa and -0.50 MPa according to the irrigation day).

Gas exchange measurements

In field-grown plants, g_s and net photosynthesis (A_N) were measured on healthy mature leaves from 17 June to 16 September. The phenological stages were from the closing of bunch to the harvest. The measures were made per day. The total number of days in which the measures were made was twenty-one. For each sampling time and variety, eight measurements were made on different plants. These parameters were measured using a portable infrared gas analyser system (LI 6400. Li-Cor, Lincoln, NE, USA). The intrinsic water use efficiency (WUE_i) was determined as the ratio between net photosynthesis and stomatal conductance. WUE_i and g_s variations were referred over the season.

All measurements were made on the sun-exposed leaves, fully expanded (usually the 6^{th} – 8^{th} on the shoot axes) using the chamber of fluorescence in different hours of the day (between 8:30 h and 20:00 h). Photon flux density (PPFD) incident on the leaves was always higher than 1500 $\mu mol.m^{-2}.s^{-1}$, which is known to be above photosynthesis saturation in grapevines (Flexas *et al*., 1998).

In every leaf, immediately after gas exchange measurement, Ψ_{leaf} was determined with a Scholander chamber (SKPM-1400, Skye Inst. Lim., U.K.).

The analysis of variance was performed using version 15.0 of the SPSS package.

RESULTS AND DISCUSSION

Stomatal sensitivity to water stress

The Fig. 1 shows morning and afternoon Ψ_{leaf} measurements over the season. Most of measures were made from the morning first hours up to midday, since we expected study the stomatal behaviour of the varieties according to three g_s ranges or stages established by Flexas *et al*., in 2004. These authors used g_s as unifier parameter of stress degree establishing a progressive answers pattern of the photosynthesis to the water stress.

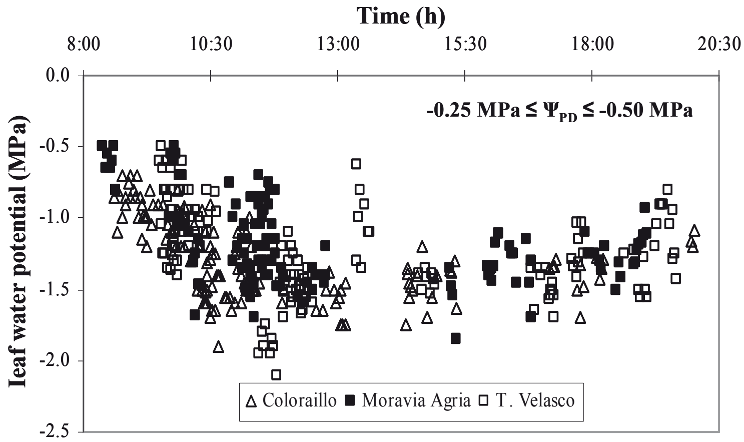

Fig. 1. Morning and afternoon measurements of Ψ_{leaf} over the season.

To compare the stomatal response of varieties with regard to water stress (considering Ψ_{leaf} as stress measure), three ranges of conductance were used: stage 1 ($g_s > 0.15$ mol.m^{-2}.s^{-1}), stage 2 (0.05 mol.m^{-2}.s^{-1} $\leq g_s \leq$ 0.15 mol.m^{-2}.s^{-1}) and stage 3 ($g_s < 0.05$ mol.m^{-2}.s^{-1}).

Moravia Agria and specially Coloraillo showed high stomatal sensitivity to water stress (Fig.2). Both varieties maintained similar Ψ_{leaf} values at different g_s levels throughout the season; mean Ψ_{leaf} values being maintained between following ranges: -1.22 MPa and -1.38 MPa in Coloraillo and -1.08 MPa and -1.29 MPa in Moravia Agria. In contrast, Tinto Velasco behaved like a less sensitive variety to water deficit with mean Ψ_{leaf} values between -1.13 MPa and -1.45 MPa. In Moravia Agria and Coloraillo Ψ_{leaf} did not decrease to a low of -2 MPa (-1.8 MPa and -1.9 MPa respectively), as compared to Tinto Velasco, where Ψ_{leaf} reached -2.1 MPa.

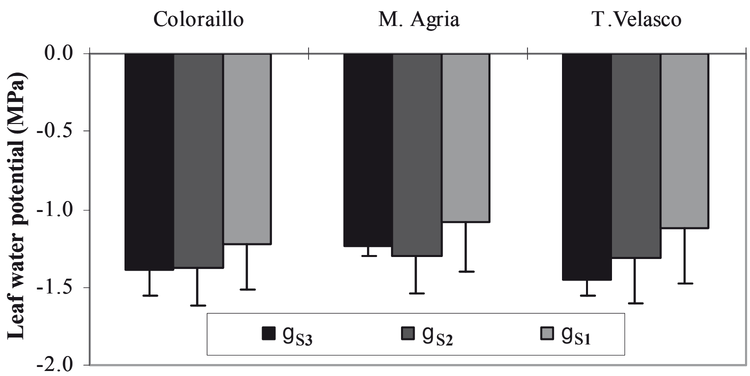

Fig. 2. Variations of leaf water potential (Ψ_{leaf}), considering three g_s ranges: range 1 ($g_{s1} > 0.15$ mol.m^{-2}.s^{-1}), range 2 (0.05 mol.m^{-2}.s^{-1} $\leq$ $g_{s2} \leq$ 0.15 mol.m^{-2}.s^{-1}) and range 3 ($g_{s3} < 0.05$ mol.m^{-2}.s^{-1})

In order to determine whether the differences observed between the varieties were significant, an analysis of variance was performed. The results are showed in the Tab.1.

When g_s declined below 0.05 $mol.m^{-2}.s^{-1}$, where the not stomatal effects (inhibition of the activity of the Rubisco, functionality of the photosystems and the majority of the components of Calvin's cycle) have great influence, Tinto Velasco was the variety with lowest mean Ψ_{leaf} values. In contrast, for g_s values above 0.05 $mol.m^{-2}.s^{-1}$ (where the stomatal effects are predominant) the lowest mean Ψ_{leaf} values corresponded to Coloraillo. Note that in this variety the number of samples for g_s values below 0.05 $mol.m^{-2}.s^{-1}$ is only three, whereas for g_s values above 0.15 $mol.m^{-2}.s^{-1}$ is 94. This may be related to anatomical and physiological differences of this variety.

Tab. 1. Average values of leaf water potential (Ψ_{leaf}) according three g_s levels. The letters indicate homogenous groups by the Duncan test (ns,*,**, non-significant, significant at P=0.05 and P=0.01 respectively) (g_s: $mol.m^{-2}.s^{-1}$; Ψ_{leaf}: MPa)

Variety	$g_{s1} > 0.15$		$0.05 \leq g_{s2} \leq 0.15$		$g_{s3} < 0.05$	
	n	Ψ_{leaf} *	n	Ψ_{leaf} ns	n	Ψ_{leaf} **
Coloraillo	94	-1.22 b	44	-1.38	3	-1.38 ab
M. Agria	76	-1.08 a	50	-1.29	8	-1.24 a
T. Velasco	55	-1.13 ab	69	-1.31	12	-1.45 b

Water use efficiency

WUE_i depends on complex interactions between environmental factors and physiological mechanisms such as stomatal behaviour, photosynthetic capacity and leaf and plant anatomy (Schultz, Stoll, 2010). In this paper, Tinto Velasco behaved like the most efficient variety in the three g_s ranges (Fig. 3). In contrast, Coloraillo was the least efficient of all three varieties (Tab.2), except for g_s values below 0.05 $mol.m^{-2}.s^{-1}$, where WUE_i values were lightly higher than Moravia Agria (maybe to the short number of samples of this variety in this g_s range). This was due to the fact that this cultivar kept stomata open, even in hours of the day when the water deficit was more acute, and was therefore a less efficient cultivar in terms of water use.

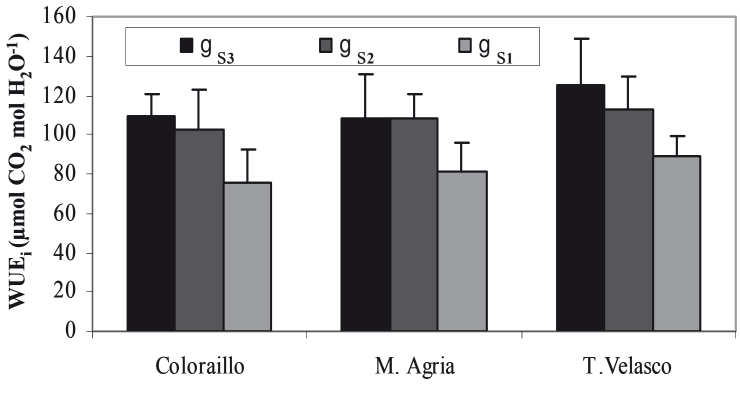

Fig. 3. Variations of intrinsic water use efficiency (WUE_i), considering three g_s ranges: range 1 ($g_{s1} > 0.15$ $mol.m^{-2}.s^{-1}$), range 2 (0.05 $mol.m^{-2}.s^{-1} \leq g_{s2} \leq 0.15$ $mol.m^{-2}.s^{-1}$) and range 3 ($g_{s3} < 0.05$ $mol.m^{-2}.s^{-1}$)

The Fig. 4 shows the relationships between WUE_i and g_s for each cultivar. In all varieties, the smaller g_s, the higher is the WUE_i. It is observed that Tinto Velasco exhibit a higher WUE_i than Moravia Agria and Coloraillo at same g_s values. Coloraillo was the variety that showed highest maximum g_s values (0.45 $mol.m^{-2}.s^{-1}$) whereas Tinto Velasco displayed lowest maximum g_s values (0.28 $mol.m^{-2}.s^{-1}$).

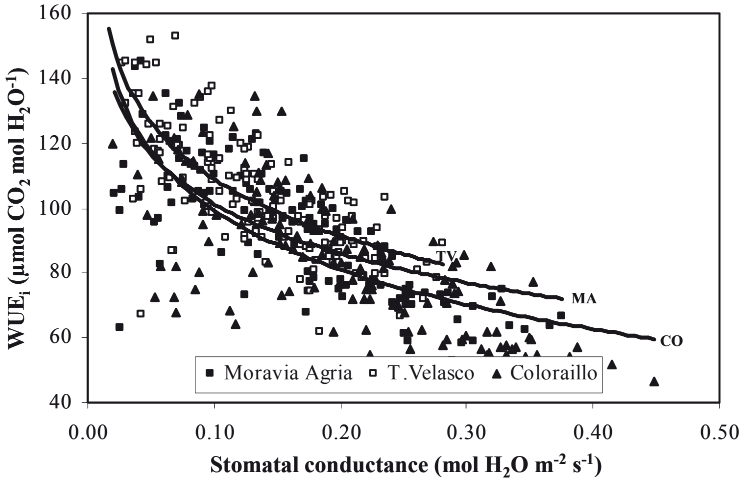

Fig. 4. Relations between intrinsic water use efficiency (WUEi) and stomatal conductance (g_s). The lines represent the adjustments for each cultivar.

The results showed significant differences between fitting lines for g_s values higher than 0.05 $mol.m^{-2}.s^{-1}$ (Table 2).

Tab. 2. Average values of intrinsic water use efficiency (WUE_i) according three g_s levels. The letters indicate homogenous groups by the Duncan test (ns,**, non-significant, significant at P=0.01) (g_s: $mol.m^{-2}.s^{-1}$; WUE_i: $\mu mol.mol^{-1}$)

Variety	g_{s1} > 0.15		0.05 < g_{s2} < 0.15		g_{s3} < 0.05	
	n	**WUE_i ****	n	**WUE_i ****	n	**WUE_i ns**
Coloraillo	94	75.11 a	44	102.79 a	3	109.14
M. Agria	76	81.40 a	50	107.96 ab	8	107.67
T. Velasco	55	89.28 b	69	112.52 b	12	124.67

CONCLUSIONS

Improving water use efficiency in grapevines is essential for vineyard sustainability because predicted future climate conditions indicate a reduction of water availability for both human and agricultural consumption. A possibility of increasing WUE_i would be based on a better control of water losses by improving of stomatal control; it could be achieved by genetic engineering and biotechnology.

In this paper is presented once again that there are differences of stomatal behaviour between grapevine varieties. This suggest that a precise study of minority grapevine varieties in terms of stomatal control and WUEi is urgent because many of them can disappear in a few years, without knowing them from a physiological point of view, and however they would be able to help us to improve varieties in the future

BIBLIOGRAPHY

Bota J., Flexas J., Medrano H., 2001. Genetic variability of photosynthesis and water use in Balearic grapevine cultivars. *Annals of Applied Biology*, 138: 353-361.

Flexas J., Escalona J.M., Medrano H., 1998. Down-regulation of photosynthesis by drought under field conditions in grapevine leaves. *Australian Journal of Plant Physiology*, 25: 893-900.

Flexas J., Bota J., Cifre J., Escalona J.M., Galmés J., Gulías J., Lefi E-K, Martínez-Cañellas S.F., Moreno M.T., Ribas-Carbó M., Riera D., Sampol B., Medrano H., 2004. Understanding down-regulation of photosynthesis under water stress: future prospects and searching for physiological tools for irrigation management. *Annals of Applied Biology*, 144: 273-283.

Gómez del Campo M., Baeza P., Ruíz, C. Lissarrague J.R., 2004. Water-stress induced physiological changes in leaves of four container-grown grapevine cultivars (*Vitis vinifera* L.). *Vitis*, 43: 99-105.

McDonald A.J.S., Davies W.J., 1996. Keeping in touch - responses of the whole-plant to deficits in water and nitrogen supply. *Advances in Botanical Research,*22: 229-300.

Medrano H., Flexas J., 2004. Respuesta de las plantas al estrés hídrico. In: La ecofisiología vegetal. Una ciencia de síntesis. Reigosa M.J., Pedrol N., Sánchez A. International Thomson Editores Spain. Madrid. 253-286.

Montero F. J., Brasa A., 1998. El viñedo en Castilla-La Mancha ante el siglo XXI. Ediciones de la Universidad de Castilla-La Mancha. Colección Ciencia y Técnica, nº 23: 15-36.

Ojeda H., Lebon E., Deis L., Vita F., Carbonneau A., 2005. Stomatal regulation of mediterranean grapevine cultivars in drought situations of the southern of France. Preliminary results. *Proceedings of GESCO*. 23-27 august 2005, Geisenheim, Germany, 173-179.

Schultz H. R., 1996.Water relations and photosynthetic responses of two grapevine cultivars of different geographical origin during water stress. *Acta Horticulturae,* 427: 251-266.

Schultz H. R., 2003. Differences in hydraulic architecture account for near-isohydric and anisohydric behaviour of two fiel-grown *Vitis vinifera L*. cultivars during drought. *Plant, Cell and Environment,* 26: 1393-1405.

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