# Genetic variation of plant water status, water use efficiency and grape yield and quality in response to soil water availability in grapevine (*Vitis vinifera* L.)

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#### Abstract

The variability on water use efficiency was evaluated in a collection of 22 grapevine cultivars growing in an experimental farm near Palma. Gas exchange parameters (net photosynthesis, stomatal conductance and transpiration) were measured in leaves four times from May to August. Also, water relation parameters (soil water potential, stem water potential), and grape yield and quality were also analyzed. The results shown that intrinsic water use efficiency (WUE, relation between net photosynthesis and stomatal conductance) measured in grapevine ranged from 42 to 78 µmol mol<sup>-1</sup> in well watered plants, but mostly of the cultivars shown values around 60  $\mu$ mol·mol<sup>-1</sup>. However when water deficit was progressively imposed, WUE increased and finally, raised values up to 150 µmol mol<sup>-1</sup>. Under those conditions of very low soil water availability (soil water potential of -1,5 MPa), the WUE ranged from 72  $\mu$ mol·mol<sup>-1</sup> (Macabeo cultivar) to 156  $\mu$ mol mol<sup>-1</sup> (Argamusa). The plant water status measured as stem water potential (Ystem) under severe water stress conditions, ranged from -0.97 to -1.67 MPa, depending of the cultivar. Interestingly, the cultivar Macabeo showed the lowest WUE and the highest *Ystem* (-0.975 MPa). Also, this cultivar presented the highest yield (fruit production per plant). On the opposite, Argamussa was the cultivar with highest WUE under water stress, because of a higher stomatal adjustment under those conditions, maintaining high net photosynthesis rates. This cultivar also showed a very low stem water potential (-1.48 MPa). The higher capacity of carbon fixation of this cultivar under water stress, was reflected in a high plant yield (7.8 Kg grape per plant), however sugar concentration in must was very low. Certain co-relation was obtained between leaf carbon fixation and total yield and sugar content in must.

# **INTRODUCTION**

WUE is always a balance between biomass gain (biomass Kilos or harvest or mols of assimilated CO<sub>2</sub>) and water losses (m<sup>3</sup> of water used or mols of transpired water). Consequently, WUE improvement can be illustrated (Figure 1) on the basis of the curves representing the relationships between photosynthesis and stomatal conductance (A vs.  $g_s$ ). The represented curves correspond to higher (B) and lower (A) leaf photosynthetic capacity (Flexas *et al.*, 2010).

As can be seen in figure 1, changing from G1 to G2 (for instance by irrigation) photosynthesis will increase but WUE will be lower. Contrarily going from G1 to G3 (irrigation restrictions or by water competition with cover crops) will lead to a lower photosynthesis but increased WUE. To jump from G1 to G4 implicates to increase WUE on the basis of a higher photosynthesis capacity. This could be achieved by some cultural practices already in use, such as careful control of diseases, high leaf capacity by generous nutrient availability, etc. but usually, this superior photosynthetic capacity is achieved by genetic improvement. In that way, there is an early work of our group (Bota *et al.*, 2001) on the genetic variability of WUE in grapevines.

In order to increase WUE it is necessary to account for different aspects including socioeconomic, agronomic and physiological. The improvement of crop WUE will be determined by the cultivar, and specially the combination between rootstock and cultivar. The influence of plant material on the key physiological processes related with WUE, water absorption, detection of stress by roots, water transport, (Alsina *et al.*, 2007; Lovisolo and Schubert, 2006), hydraulic and chemical signals (Christman *et al.*, 2007) and leaf gas exchange (Flexas *et al.*, 2007; Bota *et al.*, 2001), have been previously demonstrated. All these processes and those related with fruit ripening are modified by environmental conditions such as temperature, humidity, radiation(Jeong *et al.*, 2004) or water availability (Antolín *et al.*, 2003; Escalona, 2003).

The present study intends to investigate the genetic variability in water use efficiency (WUE) and to determine some dependence between WUE and physiological and agronomical parameters.

# **MATHERIAL AND METHODS**

#### 1. Plant material and treatments

The study was carried out in 2009, in an experimental vineyard of Majorcan government in which a large grapevine cultivars collection is present. For this experiment, sixteen local cultivars and six foreign cultivars were selected based on phenological and agronomical aspects (table 1). The plants were 10 years old grafted in 110-R rootstock, and trained at bilateral cordon system. Ten plants per cultivar were used for the experiment.

Irrigation was applied only at the beginning of flowering. Afterwards, no irrigation was applied until harvest time. The soil water content was monitored by the measurement of soil matricial potential by psycrometers (Wescor Sci. Inc., USA), at 30 and 60 cm depth of soil (Fig. 2).

#### Specific leaf mass and plant water status

Specific leaf mass was calculated as dry weight (g)/ leaf area  $(m^2)$ . Four measurements were done for each cultivar in three different times during summer. The plat water status was established at, ripening and harvest time, by the measurement of the

stem water potential at midday ( $\Psi$ stem), in four plants per cultivar, using a Scholander chamber (Soil Moisture Equipment corp., USA).

#### Gas Exchange measurements

Leaf net photosynthesis (A), stomatal conductance  $(g_s)$  and transpiration rate (E) were measured in six leaves per cultivar in four different times: flowering (M1), veraison (M2), ripening (M3) and harvest (M4). Measurements were done between eight and ten hours (solar time) using an IRGA open system Li-6400 (Li-Cor Inc., USA). All measurements were done at saturated light (1500 mmol m<sup>-2</sup> s<sup>-1</sup>) and at CO<sub>2</sub> concentration of 400 mmol CO<sub>2</sub> mol<sup>-1</sup> air.

#### Water use efficiency (WUE) determination

Water use efficiency was calculated at leaf level using two different techniques: **1.** Instantaneous measurements of gas exchange parameters using an IRGA open system Li-6400 (Li-Cor Inc., Nebraska). Intrinsic water use efficiency  $(A_N/g_s)$  was calculated from leaf measurements of net photosynthesis  $(A_N)$  and stomatal conductance  $(g_s)$ .

2. A determination of C<sup>13</sup> isotopic discrimination coefficient in dry mass as an estimation of integral WUE along the phenologic cycle. For these measurements, leaf samples were collected in two different times: veraison (M2) and harvest (M4). Leaf samples were dry at 70°C for 48 hours and milled for getting a fine dust sample for its posterior isotopic analysis of the rationC<sup>12</sup>/C<sup>13</sup>. Samples were burn in an elemental analyzer (Thermo, Bremen, Germany); and the CO<sub>2</sub> were separated by chromatography and injected directly in a isotopic coefficient mass spectrometry continuous flux system (Thermo Finnigan Delta Plus, Bremen, Germany). Sample of peach leaf was used as standard. The  $\delta^{13}C$  calculation was made as follow:  $\delta^{13}C_{sample}$  (‰) = (R<sub>sample</sub>/R<sub>standard</sub> - 1) × 1000 (Farquhar and Richards, 1984); R<sub>sample</sub>/R<sub>standard</sub> is referred to Pee De Belemnite (PDB) standard.

### Plant production and grape quality

Yield (grape production (kg)/plant ), and number of clusters, were measured at harvest in 6 plants per cultivar. Three samples of 100 berries were randomly taken from the total grape production of the six plants. The grape weight was measured and sugar content (baumé), total acidity and pH were measured in must. During winter, pruning weight of the same plants was determined. Afterwards, Ravaz index was calculated as the ration yield (kg) / pruning weight (kg) for each plant per cultivar.

#### **RESULTS AND DISCUSSION**

#### Cultivar response to progressive soil water depletion

The progressive water stress caused an important increase of specific leaf mass, increasing by 30% even more, from veraison to harvest time. Under these conditions, some cultivars showed only moderate decline of water status (defined by stem water potential) even under severe limitation of soil water availability (table 1).

Specific Leaf mass (SLM) and stem water potential ( $\Psi$ stem) showed to be cultivardependent parameters, ranging from 77 to 108 g cm<sup>-2</sup> and -0.98 to -1.70 MPa at harvest time, respectively.

Plant productivity, in terms of plant yield and sugar content in must, is also very dependent on the cultivar (table 1).

#### Water use efficiency variability

A high correlation was found between intrinsic water use efficiency (WUE) and stomatal conductance, regardless of the cultivar. (fig 3A). This correlation has been described before in grapevine (Bota *et al.*, 2001, Flexas *et al.*, 2002; Pou *et al.*, 2008; Zsófi *et al.*, 2009). Based on this relation it can be concluded that WUE can be strongly increase (from 40 to 120 CO<sub>2</sub>/mol H<sub>2</sub>O) under a moderated water stress ( $g_s$  around 0.1 mol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>). Under severe water stress conditions ( $g_s < 0.1$  mol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), the cultivar factor plays an important role. Especially interesting would be all those cultivars that below this value continue to increase their water use efficiency even in these severe drought conditions. The highest WUE value reached corresponded to the variety Giró Ros.

WUE show certain dependence with carbon Isotopic discrimination ( $\delta^{13}$ C), measured in leaf at different times during ripening, representing a good parameter of plant water status (fig 3B). The  $\delta^{13}$ C analysis can be considered an integral measurement of WUE during the period of biomass formation (Farquhar and Richards, 1984). The results show variations between 2-3 ‰ of  $\delta^{13}$ C from veraison to harvest (Figure 3B). Similar variations of  $\delta^{13}$ C has been found in other grapevine cultivars under water stress conditions (de Souza *et al.*, 2003; Chaves *et al.*, 2007; Pou *et al.*, 2008), The correlation between both parameters reinforces the validity of both assessing the efficiency at leaf level. Despite the interest of both parameters (A/g<sub>s</sub> and  $\delta^{13}$ C), both have limitations for estimating the efficiency at the level of whole plant or crop.

In general, higher plant production (expressed as yield x grape sugar content), corresponded to lower WUE (A/g<sub>s</sub>). However, Argamussa cultivar presented a high production (7.85 kg per plant ) joint with WUE near to 160  $\mu$ mol CO<sub>2</sub>/mol H<sub>2</sub>O at harvest time. Also sugar content in must was very different depending of the cultivar (table1). Leaf net photosynthesis rate measured at the end of ripening can be considered a good reference of plant yield and grape sugar content at harvest (fig 3D).

#### CONCLUSIONS

In summary, according with the presented data, it seems an evidence that there is a genetic variability of water use efficiency (WUE). Also genes determine the plant productivity and some quality parameters. Under moderate water stress, Argamussa showed high values of both, WUE and plant production. On the other hand, Giró Ros, showed high values of WUE and sugar content in must.

However, in some cases, dependence between WUE and physiological and agronomical parameters appears to be independent of the cultivar.

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# **Tables**

Table 1. Specific leaf mass at veraison, ripening and harvest, and stem water potential and grape yield, Ravaz Index and sugar content at harvest time.

		SLM(veraison) SLM (harvest)		Ystem	Grape yield	Ravaz	Sugar
	CULIIVAR	(gm-2)	(gm-2)	(MPa)	(Kg)	Index	(Baumè)
Local Cultivars	Argamussa	62,40±,57	93,42±4,45	-1,49±0,12	$7,86\pm0,50$	7.53±1,26	9,95±0,38
	Callet	56,53±2,82	85,54±1,93	$-1,70\pm0,07$	3,61±0,40	5.80±0,44	11,50±0,33
	Callet blanc	72,10±2,68	94,59±1,97	$-1,54\pm0,02$	4,74±1,02	4.56±0,99	11,55±0,13
	Escursach	56,66±3,97	84,95±5,38	-1,29±0,09	3,48±0,29	$2.35 \pm 0.37$	$11,40\pm0,10$
	Espero de gall	65,81±3,33	87,58±2,38	-1,30±0,09	$2,08\pm0,30$	$1.54 \pm 0.16$	12,95±0,12
	Galmeter	75,43±1,28	100,72±2,04	-1,45±0,11	4,26±0,24	$5.20 \pm 0.68$	12,90±0,10
	Giro ros	66,48±2,56	95,17±2,92	-1,64±0,08	$2,62\pm0,45$	$1.52 \pm 0.17$	15,10±0,13
	Gorgollasa	65,17±1,61	82,25±2,95	-1,30±0,04	$0,82\pm0,11$	$0.56\pm0,10$	12,2±0,18
	Malvasia	54,11±2,01	77,95±5,98	-1,43±0,03	1,27±0,16		14,05±0,13
	Manto Negro	59,43±1,74	78,82±4,51	-1,24±0,09	$0,66\pm0,10$	0.71±0,32	15,20±0,39
	Moll	67,62±3,33	97,51±1,69	$-1,28\pm0,08$	6,94±1,04	4.47±0,49	11,60±0,33
	Sabater	70,45±2,85	100,13±2,99	-1,38±0,06	5,32±0,62	$6.06 \pm 0,62$	12,15±0,19
	Valent blanc	53,50±1,88	106,26±4,07	$-1,30\pm0,02$	$2,94{\pm}0,49$	3.68±0,59	11,95±0,24
	Valent negre	49,33±1,77	100,72±3,35	-1,68±0,07	6,30±0,58	$4.42\pm0,52$	10,70±0,23
	Vinater blanc	72,89±,57	108,89±5,94	-1,26±0,06	3,61±0,29	$3.42 \pm 0,21$	13,20±0,14
	Vinater tinto	61,69±3,80	96,34±5,15	-1,43±0,14	3,66±0,79	5.59±1,49	11,45±0,29
Foreign Cultivars	Cabernet	54,31±1,90	81,74±4,91	-1,41±0,07	3,68±0,81		14,05±0,13
	Garnacha	65,77±2,91	93,42±2,74	$-1,30\pm0,05$	6,91±0,84		$13,45\pm0,30$
	Macabeo	62,79±1,95	88,46±3,18	$-0,98\pm0,05$	8,96±0,54		$10,80\pm0,44$
	Merlot	60,35±1,47	97,80±3,64	-1,39±0,11	3,46±0,47		14,95±0,32
	Siraz	-	85,24±5,20	-1,30±0,07	5,41±0,43		13,05±0,16
	Tempranillo	62,36±3,04	85,54±3,07	-1,34±0,07	5,73±1,22		12,45±0,13
	Cabernet	54,31±1,90	81,74±4,91	-1,41±0,07	3,68±0,81		14,05±0,13

# **Figures**



Fig.1 Relationship between leaf Photosynthesis and stomatal conductance, with indications of possible ways to modify the water use efficiency (Parry et al 2005)



Fig.2. Change of soil water availability in two different depths, during summer time (2009)



Fig.3. Relationship between intrinsic water use efficiency (WUE) and stomatal conductance (A), Carbon Isotopic composition (B), plant yield x and sugar content in must (C). Relationship between photosynthesis rate and plant yield x and sugar (D). Circles represent local cultivars and squares foreign cultivars. Dark gray: flowering; gray: veraison; white: ripening; black: harvest