BALEARIC VARIETIES OF GRAPEVINE: STUDY OF GENETIC VARIABILITY IN THE RESPONSE TO WATER STRESS

J. BOTA, J. FLEXAS, B. SAMPOL, and H. MEDRANO

Institut Mediterrani d'Estudis Avançats (CSICUIB). Departament de Biologia Ambiental, Universitat de les Illes Balears. Carr. Valldemosa, Km. 7,5; 07071 Palma de Mallorca. Spain.

ABSTRACT

The photosynthetic characteristics of twenty varieties of grapevine (*Vitis vinifera* L.) from Mallorca (Balearic Islands, Spain) and two widespread varieties, Cabernet Sauvignon and Chardonnay were studied under irrigation as well as in response to drought. Despite of the common origin of these cultivars, high variability was found for several photosynthetic characteristics under irrigation. Interestingly, these variations were significant in gas-exchange parameters (net CO_2 assimilation, stomatal conductance and intrinsic water use efficiency). Some varieties presented high carbon assimilation at high water loses' expense, whereas others were water-saving even under irrigation, which was accompanied by low CO_2 assimilation. Escursach was found to be an interesting variety, presenting high carbon assimilation at time with low water consume.These varieties also showed different responses to drought, which allowed to classify them in two main groups: "alarmist" varieties, which showed strong reductions of stomatal conductance in response to relatively low decreases of leaf water potential, and "luxurious" water consume varieties, showing low reductions of stomatal conductance under water stress.

INTRODUCTION

Genetic variability in grapevine has been studied mainly in respect to yield and quality related trays as well as in morphological characteristics, used for cultivar differentiation and identification. The characterisation of physiologic capacities of different genotypes under different climates is less frequent. However, it is well established that a deeper understanding of physiological characteristics can improve our capacity to predict cultivar performance in any environment (Schultz, 1996a). Soil water deficit is the main environmental constrain for vine growth and grape production under Mediterranean conditions as well as a main factor to control grape growth and quality (Matthews *et al*., 1990).

Variations in photosynthesis and transpiration rates provide a quickly way to characterise the plant response to water stress. For this reason, the study of genetic variability in these parameters can be an important source of information to evaluate the effectiveness in the adaptation to drought environments and also to appreciate the particular capacities of each variety to perform in arid environments. In grapevine, variations in such characteristics have been described in a limited number of varieties (Chaves *et al*., 1987; Delgado *et al*., 1995; Schultz, 1996a). In previous works, we have shown differences in water relations and photosynthetic characteristics Manto Negro, a Mallorcan cultivar reputed as drought tolerant and Tempranillo, a cultivar more sensible to water stress (Delgado *et al*., 1995; Barceló *et al*., 1998; Escalona *et al*., 1999a).

The aims of this work were to study genetic variability of physiological characters in 22 Balearic cultivars of grapevine grown under optimal conditions, to compare the effects of water stress in order to identify the most well-adapted genotypes and to evaluate the variety of drought responses.

MATERIALS AND METHODS

We studied 20 Balearic cultivars and 2 widespread cultivars (Chardonnay and Cabernet Sauvignon). Experiments were carried out with potted plants in its first growth year during August and September 1999. All plants were obtained by direct rooting of 0.3 m. shoots obtained from the grapevine germplasm collection of the "Estación experimental Rancho de La Merced" in Jerez (Spain).

The rooted shoots were planted in pots of 30 litres capacity with organic substrate and maintained at field capacity by daily watering, and fertilised with nutrient commercial solution (Fertiluq (UQSA)) once a week. Plants were grown outdoors in the experimental field of the University of Balearic Islands (UIB). When plant shoots were about 1,5 m high, the measurements were developed. To study the response to water stress, irrigation was stopped (drought treatment) and new measurements were made after six days without irrigation (soil moisture reduced by about 40%).

Leaf gas-exchange parameters (CO_2 assimilation rate,(A) stomatal conductance (g) were measured, under saturating light around midday, using an open-circuit gas exchange analyser (Li-6400, Li-Cor Inc., Nebraska, USA). The area of single leaves was measured with a portable area meter (ΔT Area meter, Delta-T Devices, U.K.). Dry weight of these leaves was measured after drying during 48-72 hours at 65ºC. The specific leaf weight was calculated using the expression SLW=dry weight/leaf area ($g \cdot m^{-2}$). The leaf water potential (Ψ) was measured with a Scholander chamber (Soil Moisture Equipment Corp., Santa Barbara, USA) at pre-dawn (06:00h local time).

One-way ANOVA was applied to assess the genetic variability of each parameter. When overall statistical significance ($p<0.05$) was found, differences among genotype means were established using a Duncan test following the rules of SPSS 7.5.2S for Windows. The relative variation rank value (%) was calculated as the difference between maximum and minimum value divided by the global average.

RESULTS

Under irrigation, some variation was present between cultivars in leaf area, specific leaf weight (SLW) and pre-dawn leaf water potential (Ψ_{PD}), although only SLW showed statistical significance (Table 1). The average area of the last fully expanded leaf ranged between 99 cm^2 (Sabater) and 35,3 cm^2 (Callet), and the SLW values lied between 50,41 $g \cdot m^{-2}$ (Manto Negro) and 86,47 $g \cdot m^{-2}$ (Argamusa). These values were lower than found in previous experiments in field growing older plants (Escalona *et al*., 1999b).

Table 1. *Area of a single leaf (Area, cm^2), specific leaf weight (SLW, $g \cdot m^{-2}$) and pre-dawn leaf water potential (Ψ_{PD}, Bars) of irrigated plants of twenty-two genotypes of grapevine. Results are means ± SE of three replicates.*

Cultivar	Area	SLW	Ψ_{PD}
Aleluya	87,5±23,3	69,1±2,9	-0,57±0,09
Argamusa	75,7±12,2	86,5±23,5	-0,35±0,08
Batista	50,0±10,2	78,5±8,3	-0,92±0,09
Boal	40,7±11,3	73,0±17,0	-1,33±0,38
Callet	35,3±4,6	52,3±2,1	-0,60±0,20
Calop Blanc	68,7±13,3	70,1±3,2	-0,80±0,00
Escursach	65,3±6,7	56,1±1,7	-0,25±0,03
Esperó de gall	56,0±9,5	61,7±0,8	-0,63±0,03
Fogoneu	46,0±5,7	52,1±4,2	-0,47±0,09

Gargollasa	72,3±19,5	59,6±2,0	-0,53±0,09
Giró	67,7±9,1	65,8±6,2	-0,60±0,06
Grumiere	80,5±27,4	64,1±1,6	-0,60±0,11
Malvasia	42,7±2,8	63,7±2,5	-0,53±0,12
Mancín	69,7±17,5	66,2±4,0	-0,82±0,02
Manto Negro	95,7±2,0	50,4±2,2	-0,43±0,13
Mollar	73,7±18,7	54,9±5,4	-0,80±0,36
Monastrell	61,3±7,1	64,1±4,5	-0,65±0,14
Prensal Blanc	53,0±11,7	67,9±3,3	-0,63±0,19
Quigat	62,0±7,8	71,0±9,3	-0,60±0,00
Sabater	99,0±16,3	61,8±2,9	-0,43±0,03
Chardonnay	85,7±32,3	66,5±4,5	-0,63±0,19
Cabernet Sauvignon	53,0±8,0	68,3±6,2	-0,37±0,03
Rank value (%)	96,97	55,77	175,32
Statistical significance	NS	***	NS

Statistical significance of differences between cultivars are given as: *(P<0.05), **(P<0.01), ***(P<0.001) and non-significant (NS). The relative variation rank value is shown as %.

The lowest value of Ψ_{PD} under irrigation conditions corresponded to the genotype Boal (-1,33 Bars) and the highest to Escursach (-0,25 Bars) (Table 1), However, these differences were non-significant. These values were similar to those found in other common varieties used in previous experiments (Flexas *et al*., 1999b), but higher than those found under field conditions (Flexas *et al*., 1998a).

Maximum CO_2 assimilation was 17,5 μmols CO_2 m^{-2} s^{-1} in Callet, whereas Aleluya was the genotype with the minimum CO_2 assimilation, (8,4 μmols CO_2 m^{-2} s^{-1}) (Table 2). The highest g was measured in Callet (463 mmols H_2O m^{-2} s^{-1}) and the minimum was found in Aleluya (174 mmols H_2O m^{-2} s^{-1}). The intrinsic water use efficiency, presented a large heterogeneity ranging from 73,2 mmols CO_2·mol de H_2O^{-1} in Calop blanc, the cultivar with highest values to 37,7 in Callet.

Although all genotypes studied were subjected to the same drought treatment, the water status achieved under drought was not uniform, showing wide variations of Ψ_{PD} among cultivars. Such heterogeneity could be due mainly to differences in total leaf area of the plants, and genetic differences in stomatal conductance under irrigation which showed a wide variation among cultivars. The effects of water stress were apparent in all genotypes but with clear differences among them. A and g suffered an important reduction as a consequence of drought. However, some varieties (Callet, Escursach, Prensal Blanc i Quigat) were less affected. Such genotypes showed also a low fall of Ψ_{PD} as consequence to drought treatment (not shown).These differences in water potential were important to determine g and A.

DISCUSSION

The relative variation rank value (%) observed in gas exchange parameters (Table 2), was around 70-80% for A and A/g, and even higher for g (around 111%), the parameter more directly related with the water consume. Both, A and g presented an important highly significant genotype effect. This is specially interesting, because of the common origin of these genotypes. The A/g, which could be considered as more selective character in a Mediterranean region (Schulze, 1988), also showed wide significant genetic variability. Considering at time physiological importance of the differences and the Duncan test associations, we have classified the cultivars in three different groups. The first, with the minimum values for each character, which includes well distinguished groups of Duncan test, usually groups a and b (Table 3); an intermediate group, containing a large number of varieties, lying between three or more groups of Duncan test; and the last group, with the maximum values, including varieties positioned in groups e and f of Duncan test.

Table 2. *Net CO_2 assimilation (A, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g, mol H_2O m^{-2} s^{-1}), and intrinsic water-use-efficiency (A/g, μmolsCO_2·mols H_2O^{-1}) of irrigated plants of twenty-two genotypes of grapevine. Results are given as means $\pm$ SE of three replicates. The means within a column followed by the same letter are not significantly different at P< 0.05 as indicated by the Duncan test.*

Cultivar	A (μmolsCO_2·m^{-2}·s^{-1})	g	A/g
Aleluya	$8,4\pm1,8^{a}$	$0,174\pm0,028^{a}$	$47,3\pm3,4^{abcd}$
Argamusa	$8,7\pm1,6^{a}$	$0,215\pm0,063^{ab}$	$44,7\pm6,9^{abc}$
Batista	$11,6\pm1,6^{abcd}$	$0,226\pm0,026^{abc}$	$51,1\pm1,8^{abcd}$
Boal	$8,4\pm0,7^{a}$	$0,209\pm0,018^{ab}$	$40,7\pm4,4^{abc}$
Callet	$17,5\pm0,4^{e}$	$0,463\pm0,017^{e}$	$37,7\pm0,6^{a}$
Calop Blanc	$9,5\pm0,9^{a}$	$0,138\pm0,034^{a}$	$73,2\pm9,9^{e}$
Escursach	$13,0\pm1,7^{abcde}$	$0,209\pm0,038^{ab}$	$63,2\pm2,8^{de}$
Esperó de gall	$10,8\pm1,7^{ab}$	$0,219\pm0,025^{ab}$	$48,7\pm2,9^{abcd}$
Fogoneu	$8,8\pm0,8^{a}$	$0,158\pm0,019^{a}$	$56,3\pm4,3^{cd}$
Gargollasa	$11,2\pm1,2^{abcd}$	$0,203\pm0,041^{ab}$	$58,0\pm7,5^{cde}$
Giró	$11,7\pm1,0^{abcd}$	$0,217\pm0,041^{ab}$	$56,1\pm6,4^{bcd}$
Grumiere	$11,3\pm1,5^{abcd}$	$0,273\pm0,036^{abc}$	$41,6\pm1,0^{abc}$
Malvasia	$8,9\pm2,4^{a}$	$0,184\pm0,071^{a}$	$52,5\pm5,5^{abcd}$
Mancín	$11,2\pm0,8^{abcd}$	$0,224\pm0,040^{abc}$	$52,5\pm6,9^{abcd}$
Manto Negro	$10,7\pm1,8^{ab}$	$0,257\pm0,008^{abc}$	$41,5\pm6,1^{abc}$
Mollar	$11,1\pm0,9^{abc}$	$0,268\pm0,017^{abc}$	$41,3\pm1,6^{abc}$
Monastrell	$14,5\pm1,5^{bcde}$	$0,327\pm0,029^{bcd}$	$44,6\pm3,6^{abc}$
Prensal Blanc	$15,7\pm1,1^{cde}$	$0,411\pm0,019^{de}$	$38,5\pm3,8^{ab}$

Quigat	10,4±0,6^{ab}	0,188±0,013^{a}	55,7±2,1^{bcd}
Sabater	16,0±2,8^{de}	0,354±0,098^{cde}	49,4±7,7^{abcd}
Chardonnay	12,4±0,6^{abcd}	0,267±0,028^{abc}	47,1±4,0^{abcd}
Cabernet Sauvignon	11,9±1,2^{abcd}	0,190±0,031^{a}	63,9±5,9^{de}
Relative variation rank value (%)	78,85	111,42	70,63
Statistical significance	***	***	**

Statistical significance of differences between cultivars are given as: *(P<0.05), **(P<0.01), ***(P<0.001) and non-significant (NS). The relative variation rank value is shown as %.

Table 3. *Classification of the 22 varieties in respect to their values of A, g, A/g under irrigation.*

A (μmols CO_2 m^{-2} s^{-1})

Group	Variation rank	Examples
1	8-11	Aleluya, Argamusa, Boal, Calop Blanc, Esperó de Gall, Fogoneu, Malvasia, Manto Negro and Quigat.
2	11-13	Mollar, Batista, Cabernet Sauvignon, Chardonnay, Gargollasa, Mancín, Giró and Grumiere.
3	13-18	Callet, Escursach, Monastrell, Prensal Blanc and Sabater.

g (mols H_2O m^{-2} s^{-1})

Group	Variation rank	Examples
1	<0,22	Escursach, Aleluya, Argamusa, Esperó de Gall, Boal, Malvasia, Calop Blanc, Gargollasa, Giró, Cabernet Sauvignon, Fogoneu and Quigat.
2	0,22-0,32	Manto Negro, Chardonnay, Batista, Mancín, Grumiere and Mollar.
3	0,32-0,46	Callet, Monastrell, Prensal Blanc and Sabater.

A/g (mmols $CO_2 \cdot$ mols H_2O^{-1})

Group	Variation rank	Examples
1	<45	Argamusa, Boal, Grumier, Prensal Blanc, Callet, Manto Negro, Mollar and Monastrell.
2	45-55	Aleluya, Batista, Chardonnay,Esperó de Gall, Malvasia, Mancín and Sabater.
3	55-75	Quigat, Calop Blanc, Fogoneu, Cabernet Sauvignon, Gargollasa, Giró and Escursach.

In respect to A (Table 3), the first group presented values below 11 μmol CO_2 m^{-2} s^{-1}. Five of the varieties studied here present higher assimilation rates than two internationally cultivated control varieties. In respect to g (Table 3), the first group comprises the genotypes which showed values lower than 0,22 mol H_2O m^{-2} s^{-1}. In general, this was in coincidence with the group of lowest values of photosynthesis. The most remarkable exception within the group of varieties with high rates was Escursach which presented high values of A with low values of g.

In which respect to A/g (Table 3), the group with lowest values (below 45 μmols $CO_2 \cdot$mols H_2O^{-1}) includes varieties with high, intermediate and low values of A. Varieties which, according to previous literature, should be included in this group with low water use efficiency are Riesling (Downton *et al*., 1987; Flexas *et al*., 1999b), Tempranillo (Flexas *et al*., 1998a) and Chardonnay (Flexas *et al*., 1999b), although in the present study Chardonnay lied within the intermediate group. Genotypes with maximum values of A/g (between 55 and 75 μmols $CO_2 \cdot$mols H_2O^{-1}) showed intermediate or low net photosynthesis rate, except Escursach, which, as indicated before, had high net photosynthesis rate together with relatively low stomatal conductance. These characteristics make this variety specially interesting for semi-arid conditions, suggesting a high capacity for growth with low water availability. Photosynthesis and water economy under water stress.

Because the different cultivars did not reach an uniform water status after the drought treatment, we have established three Ψ_{PD} intervals (Table 4) in order to distinguish cultivar dependent responses to drought. The first interval includes varieties which presented values of Ψ_{PD} between 2 and 2,5 Bars under drought (Table 8). Within such interval there are cultivars with a moderate response to drought, showing low decreases of A and g like Callet and Prensal Blanc. These varieties had a low A/g (Table 3), and they did not improve such efficiency when they were under drought. This kind of behaviour could be classified as "luxurious", as the plants do not reduce the water consumption when this become scarce. Also remarkable was Escursach, because maintained a high A with a low g, resulting in a high A/g for both treatments. Within the same interval of $\Psi_{PD,}$ a second group of varieties responded strongly to drought. Fogoneu reduced g more than 75% showing a low A/g under irrigation but increasing this ratio even at low levels of drought. This other kind of behaviour could be classified as "alarmist", as little decreases of water availability induces strong water-saving responses. According to Schultz (1996a), the varieties which adopted a "luxurious" behaviour tend to maximize the production but they do not survive under prolonged drought; the varieties which adopted an "alarmist" behaviour are often less productive, but more resistant to drought. The same differentiation can be done for the interval -3 a -3,5 Bars. Whereas Mollar practically did not suffer reduction of A and g (lower than 20%), Giró, Grumier, Mancín and Manto Negro were strongly affected (more than 50% of reduction).

Table 4. *Classification of the 22 varieties in respect to their adjustment of stomatal conductance to drought. The varieties were classified in three main groups according to the values of pre-dawn Y achieved under drought. For each of these three ranges, the varieties were divided in two different behaviours: those reducing stomatal conductance by less than 40% in respect to irrigation values ("Luxurious"), and those reducing stomatal conductance by more than 40% ("Alarmists").*

Ψ under drought	"Luxurious" (Δg<40%)	"Alarmists" (Δg>40%)
-2 to2,5 Bars	Callet, Prensal Blanc, Escursach, Quigat and Batista	Sabater, Monastrell, Gargollasa and Fogoneu
-3 to 3,5 Bars	Mollar	Giró, Grumiere, Mancín and Manto Negro
< -3,5 Bars		Aleluya, Argamusa, Boal and Calop Blanc, Esperó de Gall, Malvasia, Cabernet Sauvignon and Chardonnay

Nevertheless this collection was made up of varieties belonging to the same region, significant differences were determined between varieties in net carbon assimilation rate, stomatal conductance and intrinsic water use efficiency. Callet and Prensal Blanc, which are both reputed as highly productive varieties, presented high values of A and g, and low values of A/g. Calop Blanc was the variety with the highest control of water consume under irrigation. This variability found under irrigation remains in some degree under drought. Some varieties strongly reduced the water consumption, increasing A/g as soon as water stress commences, as Sabater; but others maintained high water consume even at low water potential. Among all these varieties, Escursach is specially interesting because it joins high values of intrinsic water use efficiency with high rates of net photosynthesis under favourable conditions, and maintains this behaviour under water stress. However, this variety is not being currently cropped.

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