LEAF ULTRASTRUCTURAL AND PHYSIOLOGICAL RESPONSES DURING PROGRESSIVE DROUGHT AND RECOVERY OF *VITIS* GENOTYPES

JM Costa[1,2], RC Cerqueira[3], T Gomes[2], AR Leandro[4], O Zarrouk[2], M Pintó-Marijuan[2], V Fernández[5], M López-Carbonell[6], S Amâncio[1], MM Chaves[1,2]

[1]CBAA-Instituto Superior de Agronomia, UTL, Tapada da Ajuda, 1349-017 Lisboa, Portugal, [2]Instituto de Tecnologia Química e Biológica, UNL, Av. da República EAN, 2780-157 Oeiras, Portugal, [3] Dep. Horticultura Univ. Estadual Paulista-UNESP, [4]IFE-Sertão Pernambucano, Petrolina, Brazil, [5]Estación Experimental de Aula Dei (CSIC), Av. Montañana 1005, 50059-Zaragoza, Spain, [6]Unitat de Fisiologia Vegetal – Dep. Biologia Vegetal, Facultat de Biologia - Universitat de Barcelona

INTRODUCTION

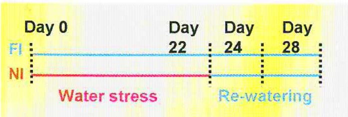
Vitis vinifera L. is a species with high genetic variability which results in variation among varieties for drought response. Our aim is to better understand leaf physiological and morphological traits influencing genotype differences in water use and drought resistance in grapevine.

We have conducted greenhouse studies in 2009 and 2010 with the varieties Syrah (SYR) and Touriga Nacional (TOU), which are relevant for Portuguese and Brazilian viticulture and were previously tested under field conditions. Plants were subjected to short term drought and recovery after re-watering.

MATERIALS AND METHODS

Plants of TOU and SYR (3-4 years old, grafted on 1103-P) were grown in 40L pots in greenhouse (38°41'N; 9°19'W), with semi-controlled ventilation (average Tair = 27.5°C and average RH of 45% in 2009; Tair = 25°C and RH=60% in 2010). Soil had median texture, slightly acid pH and 3.7% organic matter.

Treatments: 1) continuously irrigated (FI); **2)** non-irrigated (NI) for the first 16/22d (in 2009/2010) followed by irrigation until day 28. Re-watering was done when ψ_{pd}= [-10 to -12bar].

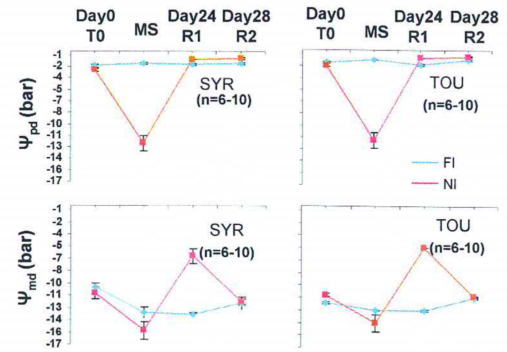


Measurements: 1) Leaf water potential; **2)** leaf gas exchange and Chl fluorescence (LiCor 6400, PPFD=1000µmol.m^{-2}.s^{-1}, Tblock=29°C, 400ppm CO_2), **3)** Chl content; **4)** xylem sap [ABA] **5)** leaf ultrastructure (discs were harvested at MS, fixed in paraformaldehyde (4%), and surface was observed with a Scanning Electron Microscope (SEM).

Observations were carried out in 2009 and 2010 at: Day 0 (no stress - T0), Day 16/22 (max. stress - MS), Day 24 (2d after restarting irrigation - R1) and Day 28 (6d after restarting irrigation - R2). The presented physiological data are relative to 2010.

RESULTS

Leaf water potential (Ψ_{pd} and Ψ_{md}) (Fig. 1)



Recovery was complete 2 days after re-watering (R_1), in both varieties.

The less negative ψ_{md} in NI at R_1 were due to stomatal closure.

A_{max} and $g_{s\,max}$ (Tab.1)

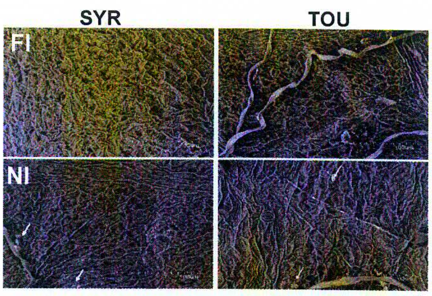
(n=5-10)

			Day 0 (T_0)	Day 22 (MS)	Day 24 (R1)	Day 28 (R2)
A_{max}	SYR	FI	10,9 ± 0,7 a	10,1 ± 0,8 * a	11,3 ± 1,0 * a	11,1 ± 0,9 a
		NI	11,1 ± 0,8 a	0,1 ± 0,1 b	4,6 ± 0,7 c	6,5 ± 1,0 c
	TOU	FI	9,3 ± 0,7 a	9,9 ± 0,4 * a	11,3 ± 1,2 * a	10,4 ± 0,8 a
		NI	9,5 ± 0,7 a	0,2 ± 0,1 b	5,7 ± 0,6 c	6,2 ± 0,8 c
g_{smax}	SYR	FI	0,280 ± 0,023 a	0,200 ± 0,004 * b	0,191 ± 0,009 * b	0,211 ± 0,006 * b
		NI	0,238 ± 0,029 a	0,009 ± 0,002 b	0,086 ± 0,012 c	0,158 ± 0,009 d
	TOU	FI	0,218 ± 0,021 a	0,184 ± 0,008 * a	0,184 ± 0,015 * a	0,194 ± 0,007 * a
		NI	0,171 ± 0,018 a	0,008 ± 0,002 b	0,079 ± 0,010 c	0,143 ± 0,013 a

Dif. letters mean sig. dif. between TOU and SYR ($p<0.05$); *sig. dif. between NI and FI treat.

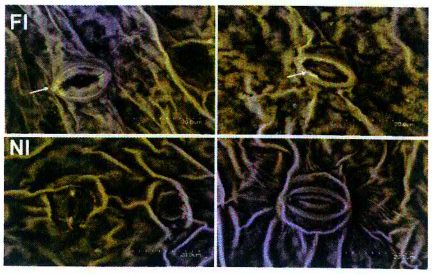
SYR and TOU show identical response of A_{max} and g_{smax} to short-term water stress. Recovery was fast but incomplete (about 60-75% of FI).

Leaf eppidermis and ultrastructure (Fig. 2)



The wax spots observed in NI leaves (arrows) suggest wax degradation/lack of synthesis.

Fig 2A. SEM images of the abaxial epidermis of leaves of SYR and TOU for the control (FI, top) and NI plants, taken at MS in 2009.



FI leaves seem to have more epicuticular and epistomatal waxes than NI at MS. Note wax accumulation in stomata of FI leaves arrows.

Fig 2B. SEM images showing stomata of SYR and TOU from FI and NI leaves, taken at MS in 2009.

Photochemical efficiency (Φ_{PSII}) (Tab. 2)

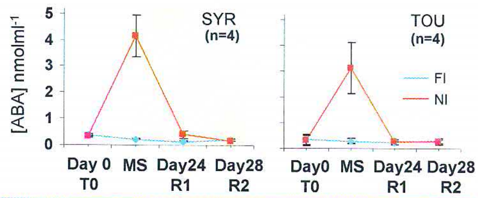
(n=5-10)

		Day 0 (T_0)	Day 22 (MS)	Day 24 (R1)	Day 28 (R2)
SYR	FI	0,16 ± 0,01 a	0,21 ± 0,02 * b	0,24 ± 0,02 * b	0,22 ± 0,02 b
	NI	0,16 ± 0,02 a	0,06 ± 0,00 b	0,09 ± 0,01 b	0,14 ± 0,02 a
TOU	FI	0,15 ± 0,01 a	0,20 ± 0,01 * b	0,21 ± 0,01 * b	0,20 ± 0,02 *b
	NI	0,14 ± 0,01 a	0,06 ± 0,00 b	0,11 ± 0,01 c	0,14 ± 0,01 ac

The partial recovery of the Φ_{PSII} can be related to Chl degradation.

Fv/Fm ≈ 0.6 for both varieties at MS.

Xylem sap ABA (Fig 3)



No differences in [ABA] were found between varieties except at R2 (TOU, NI > SYR NI)

SUMMARY

Leaf morphology: Leaves from irrigated vines had more epicuticular and epistomatal waxes than NI leaves.

Leaf water potential and gas exchange: SYR had the most pronounced decrease in ψ_{pd} and the lowest ψ_{pd} at MS in 2009, but not in 2010. Leaf gas exchange was identical in SYR and TOU. This contrasts with field results where drought was slowly imposed.

ABA: identical variation of [ABA] may explain identical kinetics of A_{max} and g_{smax} in response to short term stress-recovery cycle in SYR and TOU.

FCT Fundação para a Ciência e a Tecnologia