Hydric stress tolerance of *Arbutus unedo* L. selected trees

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Fig. 1 – *Arbutus unedo* L. (Strawberry tree)

INTRODUCTION

•*Arbutus unedo* L. (Fig. 1) is typical of Mediterranean sclerophyllous and laurel vegetation, where either frost or summer dryness are not very intense. It is an underestimated fruit tree, with different possible commercial usages from processed to fresh fruit production.

•Micropropagated clones, from selected adult trees were used to establish new orchards. It is expected, due to natural selection, that genotypes from provenances characterized by a hydric stress show greater tolerance than genotypes from more humid and cooler regions.

•In this work the hydric stress tolerance of seven *A. unedo* clones from four provenances was evaluated *in vitro* conditions.

MATERIALS AND METHODS

•Selected adult clones were *in vitro* propagated by axillary shoot proliferation (Gomes & Canhoto, 2009).

•Seven clones from different provenances were selected (Fig. 2; Tab. 1), to study the hydric stress tolerance in *in vitro* conditions.

Table 1 – characteristics of the regions of provenance of each clone

Clone	Average annual temperature	Average annual rainfall (mm)	Type of soil
AL1	12.5ºC	1200 a 1600	Lithosols & Acrisols
AL4			
ESAC_05	16ºC	800 a 1000	Podzols & Cambisols
IM6	10ºC	1600 a 2000	Lithosols
JF3	10ºC	1600 a 2000	Lithosols
HP	17.5ºC	700 a 800	Lithosols & Acrisols
PEN	12.5ºC	800 a 1000	Lithosols

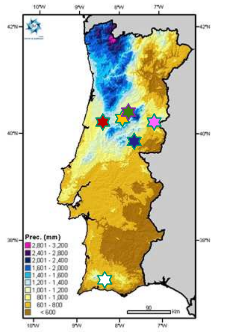

Fig. 2 – Seletced clones Provenances

•Different levels of sucrose and mannitol were added to medium culture (Tab. 2).

Table 2 – Composition of the basal medium culture.

Macro-nutrients	Micro-nutrients	Organics	BA (µM)
Anderson	MS	FS	8.9

•Sucrose and mannitol were tested and compared to the control (0.09M sucrose) to induce different levels of hydric stress (Tab. 3).

Table 3 – Tested treatments concentrations and osmotic values.

Clones		Tratamentos		
Al1; AL4; IM6; JF3; ESAC_05; HP; PEN (7 Clones)	x	Surcrose	Control	$-2.07\Psi_\pi$
			0.18M	$-4.15\Psi_\pi$
			0.29M	$-6.85\Psi_\pi$
		Mannitol	0.14M	$-3.25\Psi_\pi$
			0.49M	$-11.68\Psi_\pi$
			0.66M	$-15.58\Psi_\pi$

•Five subcultures were accomplished (14 days were used as subculture period; 3 months/total).

•The height increase, the survival rate and the proliferation were recorded.

•The *in vitro* shoots per clone and tested condition were observed to evaluate their morphological differences probably connected with the defense mechanisms to hydric stress.

RESULTS

• The hydric stress led to different behaviors in each clone, the clone from a drier and warmer zone (HP) showed the highest survival rate (Tab. 4 & 5). The less tolerant clone to hydric stress (IM6) showed a 13% survival rate, after 5 subcultures in the same medium.

Table 4 – Survival rate by clone.

Clones	Survival (%) (Average ± SE)
IM6	71.50 ± 4.49 [d]
JF3	78.50 ± 3.84 [c]
AL4	84.83 ± 2.79 [b]
ESAC_05	86.50 ± 3.26 [b]
PEN	**95.83 ± 1.52 [a]**
AL1	**96.67 ± 1.02 [a]**
HP	**99.33 ± 0.47 [a]**

Table 5 – Survival rate by subculture.

Subculture Nº	Survival (%) (Average ± SE)
5	71.43 ± 3.77 [d]
4	79.29 ± 3.08 [c]
3	90.12 ± 1.97 [b]
2	**98.21 ± 0.70 [a]**
1	**98.93 ± 0.57 [a]**

• Morphological changes: increased number of trichomas (Fig. 3) and a reduced height growth (Tab. 6), while clones from a wet and shadowed zone, sloughed completely when mannitol was tested at 0.49 and 0.66M.

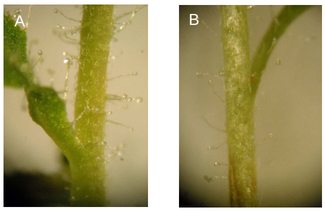

Fig. 3 – Trichome density in control medium (0.09M sucrose) of HP clone (A) vs IM6 clone (B)

Table 6 – Height increment by clone.

Clones	Height increment (mm) (Average ± SE)
IM6	0.02 ± 0.03 [d]
AL4	0.09 ± 0.02 [c]
JF3	0.10 ± 0.03 [c]
HP	0.24 ± 0.02 [b]
AL1	0.25 ± 0.02 [b]
ESAC_05	0.27 ± 0.05 [b]
PEN	**0.34 ± 0.03 [a]**

•The clone more resistant to hydric stress (HP) showed different growth after 5 subcultures: reduced height growth associated to a shoot enlargement in 0.66M mannitol (Fig. 4). Other clones increase the axillary shoot proliferation as response to osmotic stress (Fig. 5).

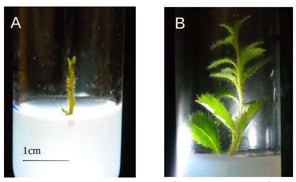

Fig. 4 – Shoot growth of HP clone, after 5 subcultures with 0.66M mannitol (A) vs control (0.09M sucrose; B)

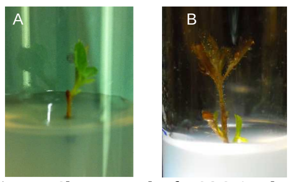

Fig. 5 – Shoot growth of ESAC 05 clone, after 3 subcultures with 0.66M mannitol (A) vs 5 subcultures 0.14M mannitol (B)

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CONCLUSIONS

• These results suggest that it is possible to predict the adaptability of a genotype to drought, considering their tolerance to the hydric stress, applied *in vitro* conditions within a short time.

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