Arbutus unedo L. selected clones conservation *in vitro* conditions

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

INTRODUCTION

•***Arbutus unedo*** **L. is a Mediterranean species. The edible fruits are used to make a spirit which represents the main income for farmers. We have started a research program aiming to select and propagate the most valuable plants based on fruit production (Fig. 1).**

•**In this work two strategies for *in vitro* conservation of selected clones of *Arbutus unedo* L. were evaluated.**

MATERIALS AND METHODS

•**Plants have been selected and *in vitro* propagated by shoot proliferation (Fig. 2).**

•**For *in vitro* conservation two conditions were tested at different periods of storage (Tab. 1). As medium culture De Fossard (1974) added of BA 8.9 µM was used. Cultures from the same genotype (360 tubes) were tested.**

•**Following the treatments the cultures were transferred to normal growth conditions and two subcultures were carried out before a new storage treatment.**

•**The multiplication rate was evaluated by the number of shoots/segments formed per test tube for further multiplication (SNX). The shoot length (SL), the number of shoots formed (SF), the proliferation (Prol) and survival rates (Surv) were evaluated.**

Table 1 – The strategies for *in vitro* conservation tested.

	The conditions tested for *in vitro* conservation	
	GCC	CS
Conditions (COND)	Growth Culture Chamber (GCC, 25/20°C, 18/6h)	Cold Storage (CS, 4°C)
Storage Periods (SP)	Periods of Storage varied between 3 to 10 months	

RESULTS

•**After the first subculture the multiplication rate was dependent upon culture conditions and the conservation storage periods (Tab. 2). The best multiplication rate (3.2) was achieved after 3 months of storage in a GCC (Tab. 3).**

•**The multiplication rate observed after 3 to 5 months was significantly higher than the multiplication rate after 6 to 10 months storage (Tab. 3).**

•**When cultures were kept in CS conditions, shoots did not elongate or proliferated, consequently the multiplication and survival rates were reduced (Fig. 3).**

•**After the 2nd subculture, no significant differences were found among treatments due to cold storage periods (Tab. 4).**

• **Thus, the multiple regression of the multiplication rate showed coefficients for the storage periods variable (SP) of -0,41 and -0,13 on the 1st and 2nd subcultures respectively (P<0.05). The multiplication rate was depend of the culture conditions (GCC *vs* CS), the number of shoot formed (SF) and shoot length (SL) (Tab. 4 & 5).**

Table 4 – Anova shows the effect of the conditions (GCC vs CS) and cold storage periods (SP) on the multiplication rate (SNX) evaluated after the 2nd subculture.

	SS	Degr. F.	MS	F	p
COND (GCC vs CS)	2.242	1	2.242	4.494	0.034387
SP (months)	4.542	5	0.908	1.821	0.106690
COND*SP	6.666	5	1.333	2.672	0.021106
Error	321.305	644	0.499		

Table 5 – The multiple regression of the multiplication rate (SNX) evaluated after the 2nd subculture.

Statistic	Summary Value
Multiple R	0,68580
Multiple R²	0,47032
Adjusted R²	0,46542
F(6,649)	96,04294
p	0,00000
Std.Err. of Estimate	0,52213

	Beta	Std.Err. of Beta	B	Std.Err. of B	t (649)	p-level
Intercept			-21.4561	4.784888	-4.48415	0.000009
COND	0.141011	0.030197	0.2066	0.044237	4.66966	0.000004
SP	-0.127481	0.030131	-0.0429	0.010143	-4.23088	0.000027
Surv	-0.005012	0.028647	-0.0917	0.523974	-0.17496	0.861167
Prol	0.000668	0.034451	0.0008	0.040259	0.01940	0.984525
SF	0.308754	0.032730	0.6595	0.069908	9.43345	0.000000
SL	0.691890	0.033833	0.0456	0.002229	20.45021	0.000000

CONCLUSIONS

•**The results suggest that the storage period should not be longer than 5 months and after the 2nd subculture the plant material may be kept for a new conservation cycle.**

•**The identification of the effect of the genotype on *in vitro* conservation response should be studied.**

•**Studies using artificial seeds and cryconservation should be implemented to reduce labor and costs, hence assuring a most effective long-term maintenance.**

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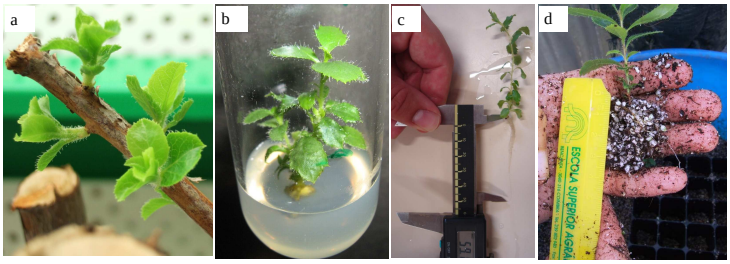

Fig. 2 - **Selected adult clones were *in vitro* propagated by shoot proliferation (Gomes & Canhoto, 2009). Epicormic shoots were used for *in vitro* establishment (a). Cultures were multiplied (b) and plantlets (c) were acclimatized in a green house and next transferred to containers (e) in nursery conditions.**

Table 2 – Effect of the conditions (GCC vs CS) and cold storage periods (SP) on the multiplication rate (SNX) evaluated after the 1st subculture.

COND	SNX (mean ± SE)
GCC	2.28 ±0.081 a
CS	1.53 ±0.051 b

SP (months)	SNX (mean ± SE)
3	2.15±0.166 a
4	2.32±0.108 a
5	2.39±0.143 a
6	1.72±0.095 b
7	1.40±0.076 c
10	1.48±0.090 bc

Table 3 – Effect of the interaction between the conditions (GCC vs CS) and cold storage periods (SP) on the multiplication rate (SNX) evaluated after the 1st subculture.

COND	SP (months)	SNX (mean ± SE)	N
GCC	3	3.17 ±0.192 a	30
GCC	4	2.60 ±0.149 b	30
GCC	5	2.87 ±0.202 ab	30
GCC	6	1.97 ±0.140 c	30
GCC	7	1.53 ±0.115 de	30
GCC	10	1.52 ±0.154 de	29
CS	3	1.13 ±0.063 e	30
CS	4	2.03 ±0.140 c	30
CS	5	1.85 ±0.143 cd	26
CS	6	1.47 ±0.115 de	30
CS	7	1.24 ±0.087 e	25
CS	10	1.45 ±0.094 de	29

Variable	Factor Loadings (Unrot Extraction: Principal cc (Marked loadings are > Factor 1	Factor 2
COND	0,647704	0,596644
SP	0,102118	-0,433396
Prol	-0,734755	0,454832
SF	-0,715852	0,520836
SL	-0,603354	-0,722287
SNX	-0,788259	0,090014
Expl.Var	2,467645	1,551759
Prp.Totl	0,411274	0,258627

COND - GCC *vs* CS
SP - storage periods
SNX - multiplication rate
SL - shoot length
SF - number of shoots formed
Prol - proliferation rate

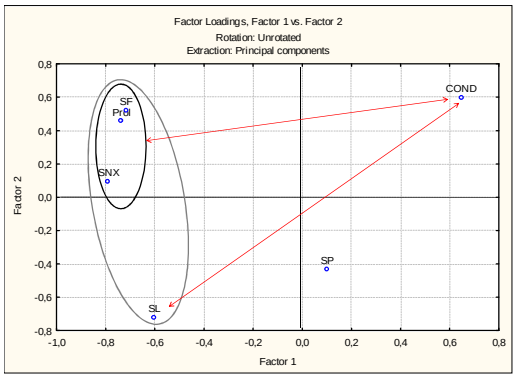

Fig. 3 – PCA analysis showing the effect of conditions tested for *in vitro* conservation.

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