In vitro conservation of *Arbutus unedo* L. selected clones using artificial seeds

Gomes, F.[1]; Pop, R.L.[1,2]; Figueiredo, P.[1]; Santos, R.[1]

1- CERNAS, Escola Superior Agrária de Coimbra, Bencanta, 3040-316 Coimbra, Portugal
e-mail: fgomes@esac.pt
2 - University of Oradea, Str. Universităţii nr. 1, 410087, Oradea, Romania

Fig. 1 - *Arbutus unedo L.* (strawberry tree) orchard

INTRODUCTION

• ***Arbutus unedo* L. grows in the Mediterranean region. The fruits are used to make a spirit which represents the main income for farmers.**

• **Adult plants from different regions have been selected, micropropagated (Gomes *et al.*, 2010) and clonal trials have been set (Fig. 1). The market demand for selected plants increased.**

• **After planting, there is a gap time (5 years) required for elite clones' identification and clonal allocation to different ecological conditions, so *in vitro* clonal conservation is crucial.**

• **Previous studies that used artificial seeds for clonal conservation showed high rates of necrosis due to phenols release. In this study, 2 culture media were used and 3 antioxidants treatments were performed.**

MATERIALS AND METHODS

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

• **To reduce the tissues necrosis observed after the artificial seeds processing, two culture media during multiplication phase were tested (Table 1), to improve the shoots' vigor .**

Table 1 – Culture media tested before artificial seeds processing

Culture Media	Macro-nutrients	Micro-nutrients	Organics	BA (µM)	Sucrose (%)
Anderson (And)	Anderson	MS	FS	8.9	3
Knop (K)	Knop	MS	FS	--	3

• **Nodal segments (5-7 mm; without leaves) were isolated and prepared to perform the antioxidant treatments (Table 2).**

Table 2 – The antioxidants treatments performed during 1 hour

Treatments	Antioxidant treatments description
H2O	Sterile distilled water
CA	Sterile solution with charcoal (CA 1%)
AO	Antioxidant solution (ascorbic acid & citric acid)
C	Control

• **Nodal segments were mixed in the culture medium added of Na-alginate (2.75%). For encapsulation they were released into $CaCl_2$ $2H_2O$ (50 mM; for 30 min.). Artificial seeds were washed 3 times in sterile distilled water and then transferred to Petri dishes.**

• **After one month (4ºC) and a reactivation period (25/20ºC, in dark, 1st week; plus 16/8h, 2nd week), different observations were performed to evaluate the survival *vs.* necrosis rates.**

• **The average time of germination (days; ∑ (NxTx)/ nº of germinated synseeds) and germination rate (conversion of syntetic seeds to shoots) were recorded.**

RESULTS

• **When Knop medium and AO or H2O antioxidant treatments were tested, shoots showed more vigor and thereafter the survival and germination rates were higher than artificial seeds previously cultured on And medium (Table 3 and Fig. 2).**

Table 3 – The germination rate (%) observed after 15 days

Cult. Media x Treatment	Germination (%) (Average ± SE)
And x AO	42.86±17.4
And x CA	52.38±17.6
And x H2O	51.39±11.9
And x C	30.56±10.9
Knop x AO	**62.50±12.5**
Knop x CA	29.17±4.2
Knop x H2O	**61.11±5.6**
Knop x C	42.00±21.0

Fig. 2 – A synseed germinated.

• **The average time of germination was dependent of the media culture (Fig. 3). When Knop medium was tested the average of time of germination was 7.5±0.38 days**

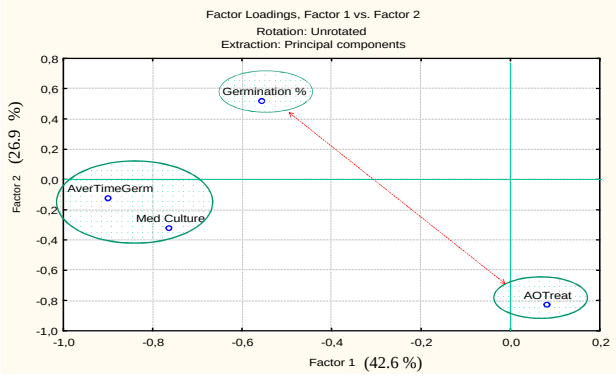

Fig. 3 – PCA analysis showing the effect of different variables on clonal conservation using artificial seeds

CONCLUSIONS

• **When nodal segments were cultured on Knop medium and antioxidant treatments were performed (AO; H2O), the artificial seeds showed more vigor and a superior germination rate.**

• **Further studies using artificial seeds should be implemented to assure a most effective long-term conservation.**

REFERENCES

Gomes, F., Simões, M., Lopes, M.L., Canhoto, J.M., 2010. Effect of plant growth regulators and genotype on the micropropagation of adult trees of *Arbutus unedo* L. (strawberry tree). New Biotechnology 27, 882-892.
Gomes, F., Canhoto, J.M., 2009. Micropropagation of strawberry tree (*Arbutus unedo* L.) from adult plants. In Vitro Cell. Dev. Biol.-Plant 45, 72-82.
Lambardi, M., Benelli, C., Ozudogru, E.A., Ozden-Tokatli, Y., 2006. Synthetic Seed Technology in Ornamental Plants. Floriculture, Ornamental and Plant Biotechnology Volume II, Global Science Books, UK, pp. 347-354.

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