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

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Arbutus unedo L. grows in the Mediterranean region. Extensive areas of *A. unedo* occur in Portugal Southern mountainous and a fragmented distribution occurs in the central and northern regions. Fruits are used to make a spirit called "medronheira", which represents the main income. The market demand for selected plants increased. Adult plants from different regions have been selected, micropropagated and clonal trials have been set. After planting, 5 years (on average) is the gap time required for elite clones' identification and clonal allocation to different ecological conditions. I*n 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 tested and 3 antioxidants treatments were performed and compared to control.

Shoots were cultivated in 2 media culture: Anderson (And) added of 8.9 µM BA *vs* Knop, without plant growth regulators. Nodal segments (5-7 mm) were isolated and prepared to perform the antioxidant treatments (for 1 H): 1) in sterile distilled water (H

$_2$O); 2) in sterile solution with charcoal (CA 1%); 3) in antioxidant sterile solution (AO; ascorbic acid & citric acid). Then nodal segments were mixed in the culture medium added of Na-alginate (2.75%). For encapsulation the nodal segments were released into $CaCl_2$ 2H2O (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), the artificial seeds were exposed to the sterile air flow, during different periods (30 min. maximum) in a laminar-flow hood, before transfer to a new fresh medium. The survival and germination rates were recorded. After 4 weeks, shoots were cultivated and the multiplication rate was evaluated. When Knop medium was tested, shoots showed more vigour and thereafter the survival rate was significantly higher than artificial seeds from nodal segments cultured on And medium. The germination was first noted on nodal segments cultivated on Knop medium ($P < 5\%$) and when AO or H_2O antioxidant treatments were tested. In these conditions it was recorded the highest multiplication rate. Further studies using artificial seeds and cryconservation should be implemented to reduce labor and costs, hence assuring a most effective long-term conservation.

Key words**:** Alginate; micropropagation; Strawberry tree; synseeds;