

PRODUCTION AND ESTABLISHMENT OF MICROPROPAGATED PLANTS

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BOOK OF ABSTRACTS

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SESSION 1- UNDERSTANDING *IN VITRO* GROWTH

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***In vitro* conservation of *Arbutus unedo* L. selected clones using slow growth storage**

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Arbutus unedo L. grows in the Mediterranean region. Its fruits are used to make a spirit called “medronheira”, which represents the main income for forest owners. Adult plants from different regions have been selected and micropropagated and clonal trials have been set in different ecological conditions. Five years after a clonal trial establishment, the clonal production was significantly higher ($557.5 \pm 5.8 \text{ Kg ha}^{-1}$) compared to seedlings ($62.6 \pm 1.2 \text{ Kg ha}^{-1}$). The clonal plants produced 8.9 times more than seedlings. The best results were observed with clonal plants when fertilized and at the best site fertility ($1192.5 \pm 70.7 \text{ kg ha}^{-1}$). Thus, the market demand for selected plants increased. However, after planting, 5 years (on average) is the gap time required for elite clones' identification and clonal allocation to different site fertility, so *in vitro* clonal conservation is crucial. In this study, different conditions for slow growth storage (SG) were evaluated during different periods (1, 3, 6 months at 4°C in the dark on gelled medium MS half strength, 0.45 μM BA). Different sucrose (suc.) and mannitol (man.) concentrations (0.16, 0.22 M) were tested and compared to control (0.09 M suc.). A reactivation period (25/20°C, in dark, 1st week; 8/16 h, 2nd week; 16/8 h, 3th week) was accomplished after the conservation periods. The survival rate was recorded after the different periods of conservation. After the reactivation period, shoot length was evaluated and then shoots were cultivated and the multiplication rate (MR) was assessed after 4 weeks. The first and second MR were recorded after the different periods of conservation to identify the best condition for SG. The best results of survival rate were achieved when sucrose was tested compared to mannitol ($P < 5\%$). The lowest survival rate was observed with man. (without significant differences between the concentrations tested). The best results for shoot length were observed with 0.09 M and 0.16 M sucrose, after 1 and 3 months (3.3 and 2.9 cm; $P < 5\%$; $N=450$). The best results for first MR were achieved with 0.09 M sucrose (2.4 ± 0.2 ; 2.1 ± 0.1 ; $P < 5\%$) after 1 and 3 months of cold storage respectively. Contrasting, when mannitol was tested the MR was inferior ($P < 5\%$), recording the best results after 1 month, with 0.16 M mannitol (0.06 ± 0.04). At the second multiplication rate, the conservation periods showed significant differences, recording the shortest period (1 month) the best results (3.7 ± 0.2 ; $P < 5\%$), when 0.22 M sucrose was tested. After 3 months of slow growth storage, the best results (for second multiplication rate) were observed at the same sucrose concentration (2.1 ± 0.2). These results show that sucrose assures *in vitro* conservation, using slow growth storage, compared to mannitol. We point out that longer periods of storage (9-12 months) are being tested to reduce labor costs, thus achieving the most effective long-term conservation.