Short-Term Conservation of *Juglans regia* L. via Synthetic Seed Technology

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Keywords: native walnut varieties; *in vitro* culture; encapsulated shoot tips; conservation period

Juglans regia L. is a crucial species as a forest tree and for its nutritional and medicinal values. However, walnut trees have consistently been subject to deliberate tree cutting and arson, which have reduced their population. For this reason, in Albania, this species is included in the list of endangered plants (EN) and thus, there is a need to find methodologies to ensure its rapid regeneration of selected lines and *ex situ* conservation. Applying *in vitro* techniques for the mass production of plants can ensure the production of a homogeneous material and allow biodiversity conservation. The encapsulation technique for creating synthetic seeds is essential for *in vitro* culture and being as an alternative to natural seeds, has potential advantages such as efficient mass production, the rapid delivery of plantlets, easy handling and transportation, increased efficiency of *in vitro* propagation in terms of space, time, and labor, and cost-effective and efficient short-mid-term storage or cryopreservation. This research, investigated the regeneration of plantlets from synthetic seeds containing shoot tips of four native walnut varieties in Albania. The walnut tree is considered one of the most recalcitrant species, thereby presenting difficulties regarding its regeneration rate. Therefore, for its *in vitro* propagation and conservation it is necessary to define the optimal culture conditions and medium that are optimal for its establishment and proliferation. Firstly, *in vitro*-derived shoot tips from walnut seedlings are encapsulated using sodium alginate. After that, the regeneration potential of the encapsulated shoot tips and the influence of incubation conditions are evaluated. The synthetic seeds were incubated at either 25º C or 8º C, with and without dehydration treatment, in 0.5 M sucrose solution for 3 h. The synthetic seeds in both temperature regimes (25º C and 8º C) develop plantlets and provide conservation potential without the need for subcultures for 4 and 3.5 months, respectively. Furthermore, all walnut varieties incubated in these conditions achieve a high regeneration rate. In conclusion, in this study, walnut zygotic embryos proved to be a good choice for tissue culture. They were also found to have better potential to develop seedlings in the DKW medium than in the other media tested. Moreover, the production and storage of encapsulated shoot tips from *in vitro* seedlings was successfully applied for the regeneration and conservation of the walnut varieties. An important finding is that the encapsulated explants showed high germination and regrowth rates, indicating potential propagation and short-term conservation applications. Specific conditions affected the shoots' shelf life and regenerative capacity. Synthetic seeds at 25º C and 8º C allowed maintaining the walnut explants for 3.5 and 4 months respectively without subcultures. These findings are promising, given the satisfactory regrowth in all tested walnut varieties after short-term storage, and the way for the application of encapsulation and storage in other *Juglans* spp. Moreover, encapsulation technology can be used in cryopreservation procedures for the long-term conservation of this species.

Reference: V. Sota, C. Benelli, M. Myrselaj, E. Kongjika & N. S. Gruda (2023) Short-Term Conservation of *Juglans regia* L. via Synthetic Seed Technology. Horticulturae 2023, 9, 559. https://doi.org/10.3390/ horticulturae9050559